GENETIC STUDIES OF POLYMORPHISM IN LABORATORY AND NATURAL POPULATIONS OF THE AUSTRALIAN PLAGUE LOCUST, *CHORTOICETES TERMINIFERA*

by

Allan Douglas Hawke

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

Allan Douglas HAWKE.

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SUMMARY

The object of this thesis was to study the colour pattern polymorphism in the Australian Plague Locust, *Chortoicetes terminifera*. This was achieved by investigating the formal genetics, followed by population studies of gene and genotype frequencies during different seasons. Other laboratory and field experiments were devised in order to explain the processes that maintain the observed variability.

Byrne's (1967a) thesis that there are nine common phenotypes in the laboratory/field (the genotypes A/A A/R being indistinguishable phenotypically) has been confirmed. For all practical purposes the genes A,N,R,T may be treated as a multiple allelic series (as postulated by Byrne (1967a)).

The mode of inheritance of the rarer porphyrica phenotype has been determined in both races. It appears as if this colour pattern is phenotypically dominant in the eastern race whilst in the western race it is recessive. No recombination between the genes of the F Locus (including porphyrica) has been detected despite an intensive investigation involving a large number of reciprocal "back-cross" matings.

Investigation of the genetics of the porphyrica colour pattern led to the discovery of the first spontaneous sex-linked gene in the Orthoptera. The phenotype is characterised by a uniformly black coloured tegmen which has been found to be recessive in character and determined by a gene located on the X chromosome.

Random mating with respect to the different colour patterns was suggested by both laboratory and field experiments. Colour pattern frequencies between males and females were found to be significantly different in seven of the 11 natural populations studied - i.e. the selective value of the genotypes varied from population to population. Cyclical selection was inferred to operate in natural populations with respect to the rubiginosa and nigrovirgata genes in a complementary way causing their frequency to fluctuate seasonally.

Of 22 comparisons available from the eleven natural populations, 16 showed a distinct excess of the 'one dominant' class (i.e. R/T, R/N, R/A). Apparently, there is a selective disadvantage of the heterozygous dominants with each other (A/N, A/T, N/T) and a similar aversion in the homozygous classes (A/A, N/N, T/T). The polymorphism seems to be maintained by the selective advantage of heterozygous over homozygous forms.

Matings were set up in order to estimate the relative selective values of the various pattern morphs in culture. Variation in the mean number of hatchlings within the homozygous and heterozygous classes was detected in the eastern race. No such difference was found in the western race.

Data from breeding experiments indicated that the percentage viability of the homozygotes in the eastern race was 82.7% of that of the heterozygous classes. In the western race the deficiency was less marked - being 96.0%. Assigning a value of 1 to the single dominant class (N/R, R/T)

the relative excesses/deficiencies were calculated to be:

	Eastern Race	Western Race
Double dominant heterozygous class (A/T, A/N, N/T)	0.94	0.96
Dominant homozygous class (A/A, N/N, T/T)	0.80	1.06
'Universal recessive' (R/R)	0.79	0.80

In a study involving the direct determination of viability of the various colour patterns from first instar to adults under controlled experimental conditions, photoperiod was found to have no effect on viability. However, differential colour pattern viability was found within and between the other experimental conditions employed.

Cytological examination of the locust revealed that the number of chiasmata per cell was generally slightly higher than the number of bivalents. At least 53% of bivalents formed have only one chiasma, whilst a minimum of 90% of all bivalents formed have two chiasmata or less. The Nolte effect which proposes a positive relationship between increased chiasma frequency and increased locust density was not confirmed. Quantitative estimation of the degree of chiasma localisation indicated that a large number of chiasmata were clustered between 81 to 100 on a 100 unit length chromosome. This pattern is more pronounced in the smaller chromosomes although it is consistent for all bivalents. Generally there was no difference in chiasma frequency or degree of localisation among the ten major colour pattern types.

CHAPTER ONE

GENERAL INTRODUCTION

Evolutionary theory is now extensive, chiefly as a result of research arising from the mathematical approach to evolution by Fisher, Haldane and Wright. These authors established the basis for population genetics by elucidating some of the factors which alter gene frequencies in populations. The expanding rate at which papers on the mathematical theory of population genetics are being published attests to the foresight of the pioneering works. Unfortunately experimental work has lagged behind the mathematical field. Of necessity then, much of the mathematical theory is based on assumptions about population structure, selective intensity, and other factors which have not been adequately investigated in natural and/or laboratory populations. As a result, different workers often hold conflicting views about the relative importance of various factors involved in the evolutionary process. For example, the method by which dominance is evolved (Fisher, 1928a,b, 1929, 1930a,b, 1931, 1934; Haldane, 1930, 1939) or even if it evolves at all (Wright, 1929a,b, 1934a,b). The relative importance of natural selection and genetic drift is another area of contention (Fisher, 1930a; Wright, 1931, 1949, 1970).

In the past 20 years a considerable amount of experimental and observational study has been undertaken specifically to investigate the accuracy of predictions advanced from mathematical theory. It has become clear that natural populations of sexually reproducing organisms

contain a large amount of genetic variability, ranging from conspicuous polymorphisms down to variation at the molecular level. Electrophoretic techniques have uncovered a vast amount of previously undetected variation in many protein systems resulting in an upsurge of the argument between the neutral mutation and pan-selectionist viewpoints (q.v. Crow, 1971). However, it should be noted that electrophoretic studies have led to misrepresentation of the true situation. Negative results usually remain unpublished, and it is the fashion to study and report only those systems and organisms which show variant isozymic forms. Nevertheless, the mechanism by which such variability is maintained has become one of the major problems of population genetics. Some criteria known to maintain genetic polymorphism have been reviewed by Karlin and McGregor (1972) and Maynard-Smith (1972).

The literature on polymorphism has become so large that it is now necessary to refer to recent summaries and reviews for an overview of the field before consulting papers of specific interest (e.g. Ford, 1940, 1953, 1965; Huxley, 1942, 1955a,b; Dobzhansky, 1951; da Cunha, 1955; Remington, 1958; Kennedy, 1961).

Quantitative characteristics such as bristle number in Drosophila have also been subjected to investigation (e.g. Fraser, 1968). The high correlation observed between parent and offspring and the efficacy of artificial selection indicate that such variation is genetically

determined. Fisher (1930a) espoused the view that the number of loci involved in determining quantitative characteristics was large and that such genes were very important in the evolutionary process. However, recent evidence indicates that while Fisher's statistical theory of quantitative variation remains valid the number of genes concerned is probably somewhat less than envisaged by him. For example, analyses of the sternopleural bristles in Drosophila and the heading time in wheat suggest that quantitative variation is mainly determined by segregation at ten or less loci, and that the genes involved act additively (Robertson, 1967).

Chromosomal polymorphisms constitute yet another intensively studied field, particularly in the Drosophila genus and related organisms where large salivary gland (polytene) chromosomes enable relatively easy study of the various inversions (Dobzhansky, 1947). In most organisms these techniques of study are not available, limiting the possibility of detecting inversions and translocations. However, the advent of the trypsin digestion method (Seabright, 1971; Chiarelli *et al*, 1972) and giemsa staining procedure (Webb, personal communication) both of which lead to differential staining of the chromosomes, may encourage more research in this area.

Before the introduction of gel-electrophoresis, the most frequently reported and investigated polymorphisms were those pertaining to conspicuous colour patterns exhibited by many organisms. Perhaps the most extensively investigated group in this category is the Lepidoptera,

where Ford (1971) and his colleagues (Sheppard, 1951 *et seq*; Clark and Sheppard, 1955 *et seq;* Cook 1961 *et seq*; Dowdeswell, 1961 *et seq*) have been the main contributors.

Genetic analyses have been completed in relatively few cases. For instance, the genetics of banding in the snail *Cepaea nemoralis* which has been studied for over 75 years is still by no means fully understood (Cain and Sheppard, 1957). In a number of cases such as the colour pattern genes in the grouse locusts (Nabours, 1929) the polymorphism is found to be accompanied by a large series of multiple alleles (or by pseudoalleles at closely linked loci). However, the reason for such a large number of alleles in most cases is not clear. Reduction of the frequency of homozygotes has been suggested as one probable selective advantage (Mayr, 1963). The organisation of a group of genes, concerned with a particular trait, into such a gene complex has been termed a 'super-gene' (Ford, 1965; Sheppard, 1969).

In such polymorphic populations the most frequent phenotype is by no means that containing a 'dominant' gene. The recessive gene in the dimorphic moth *Leucodentia bicoloria*, for instance, is far more common than its dominant allele in most districts (Suomalainen, 1941). The same is true of *Cepaea* (Cain and Sheppard, 1950), Lebistes (Haskins *et al*,1961)and the grouse locusts (Nabours, 1925) where recessive genes are more common than the dominant alleles at many localities. Why the most common allele in a series of multiple alles (or super gene) is so often the recessive is not clear.

If morph genes are selected in most cases for their physiological effect, the gene recessive for its morphological contribution to the phenotype may be dominant with respect to its physiological phenotype. Such dominance would permit the rapid spreading of a morphologically recessive gene. However, it is unlikely that this is the reason in all the cases cited above.

The purpose of this thesis has been to investigate the colour pattern polymorphism in the Australian Plague Locust, *Chortoicetes terminifera* (Walk.) utilising experimental breeding, cytogenetic and field studies. The value of such an ecological-genetic approach, which is primarily concerned with the adjustment and adaptation of wild populations to their environment, has been outlined by Ford (1971). This approach supplies the most direct means of investigating the actual process of evolution taking place at the present time. As such, ecological genetics is distinct from evolutionary genetics (which largely ignores the ecology) and population genetics (which is mainly concerned with laboratory cultures or is developed along purely mathematical lines). Some aspects of ecological genetics although not directly concerned with the evolutionary process, may be indirectly involved. For example, the genetic study of adaptation to different environments, and the distinction between races and related species of an organism. Such studies often impinge upon other areas - e.g. two current aspects of controversy, polymorphism and the evolution of dominance. These are considered in the general discussion as a direct result of the experimental work carried out in this thesis.

In the past it has proved possible to develop the work of theoretical population geneticists, enabling the construction of more realistic models, because of the ecological-genetic approach to particular problems. For example, it is only in the last decade that recognition has been paid to the fact that advantageous qualities are frequently favoured or balanced in particular environments by far greater selection pressures than had been envisaged, a discovery which has profoundly influenced evolutionary concepts (Ford, 1971). Moreover, when the genetics and ecology of local races and distinct species, whose features remain constant, are studied, an important evolutionary phenomenon is demonstrated. The stability of an organism when exposed to fluctuating extremes in any natural environment reveals that selection may also actively force the population to maintain an average type (Ford, 1971).

The relative ease of collecting field material and experimental breeding make *Chortoicetes* a good organism for study. The present thesis continues the work of Byrne (1962) on colour pattern genetics and their evolutionary significance as well as considering several related conjectural issues such as the evolution of dominance.

CHAPTER TWO

BIOLOGY

2.1 GENERAL BIOLOGY

A considerable amount of work on the general biology, ecology and life history of *Chortoicetes* has been carried out, notably by Key (1938 *et seq*), L.R. Clark (1947 *et seq*) and Clark (1965 *et seq*). Part of this Chapter summarises their work and that of other relevant studies. It is necessary to provide this background since interpretation of the studies described in the remainder of the thesis requires a familiarity with the insect.

Adults of the Australian Plague Locust *Chortoicetes terminifera* Walk.) typically have red hind tibia and transparent hind wings (Fig. 2.1). When adults take to flight the darkened area at the tip of the wing produces an impression of 'smokiness'. These characteristics distinguish *Chortoicetes* from other Acridid species.

There is a pronounced sexual dimorphism, females being about 1.7 times heavier than males. Females have four sclerotised valves at the end of their abdomen, which aid in clasping during copulation and are used in boring into the soil during oviposition. The genitalia of males are enclosed so that the end of the abdomen appears smooth and rounded, except for a small protruding triangular area on the ventral surface of the eleventh sternite.

Eggs are laid in pods (ootheca) below the soil surface (Fig. 2.2). In the pod the eggs are arranged with

FIGURE 2.1

Adult *Chortoicetes terminifera*

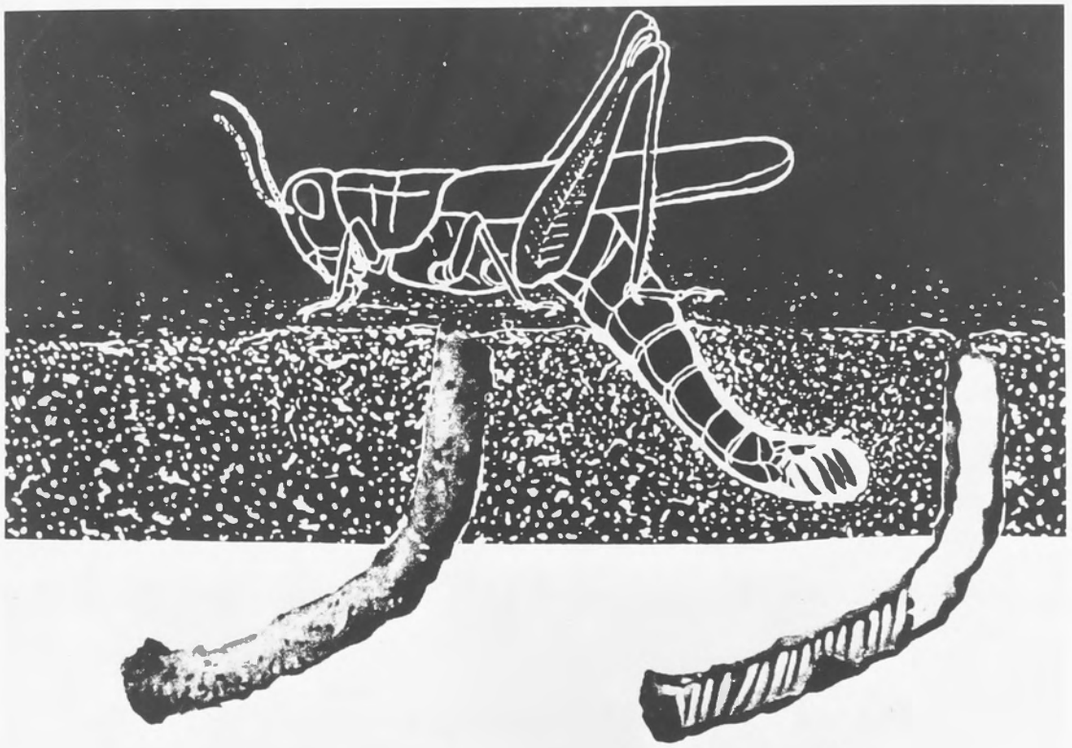
Male (left) Female (right)

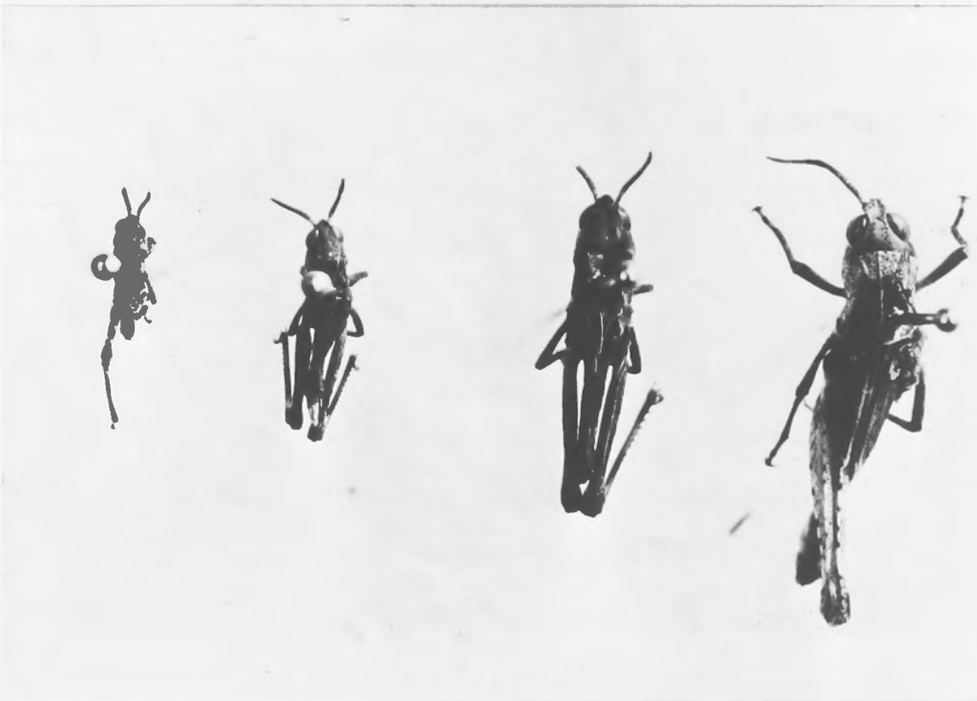
FIGURE 2.2

Egg Pod of *Chortoicetes*

FIGURE 2.3

Instar stages of *Chortoicetes*





their long axes perpendicular to the main axis of the pod and occupy the distal two-thirds of it. During oviposition the female secretes a frothy substance, which on drying serves to hold the eggs together as well as to cement the earthen wall of the hole and plug its entrance. The proximal one-third of each pod consists of dried froth allowing a relatively easy method of escape for newly hatched nymphs. A detailed description of the mechanics and female behaviour in oviposition may be found in Section 2.3.

Warmth and moisture are both necessary for egg development. Under field conditions hatching commences when soil temperatures pass a hatching threshold (Wardhaugh, 1973). Moreover, hatching is diurnal in nature, ensuring that nymphs do not hatch during the hottest part of the day or at night. Swan (1956) has reported that eggs do not develop at soil temperatures below 15^{o}C. He suggested that under field conditions, eggs usually remain dormant during the winter months and recommence development in late spring. Swan (1956) also demonstrated that eggs could remain dormant in dry soil for several months resuming development after moistening of the soil. In such eggs, growth was arrested when the embryos had completed about a quarter of their total development. The situation is even more complicated than at first thought because of the occurrence of a previously undetected embryonic egg diapause (q.v.2.7).

When the first instar emerges from the egg, it is enclosed by an embryonic membrane which it sheds within thirty minutes. The newly emerged nymph is pale creamy white, but soon becomes pigmented after the integument

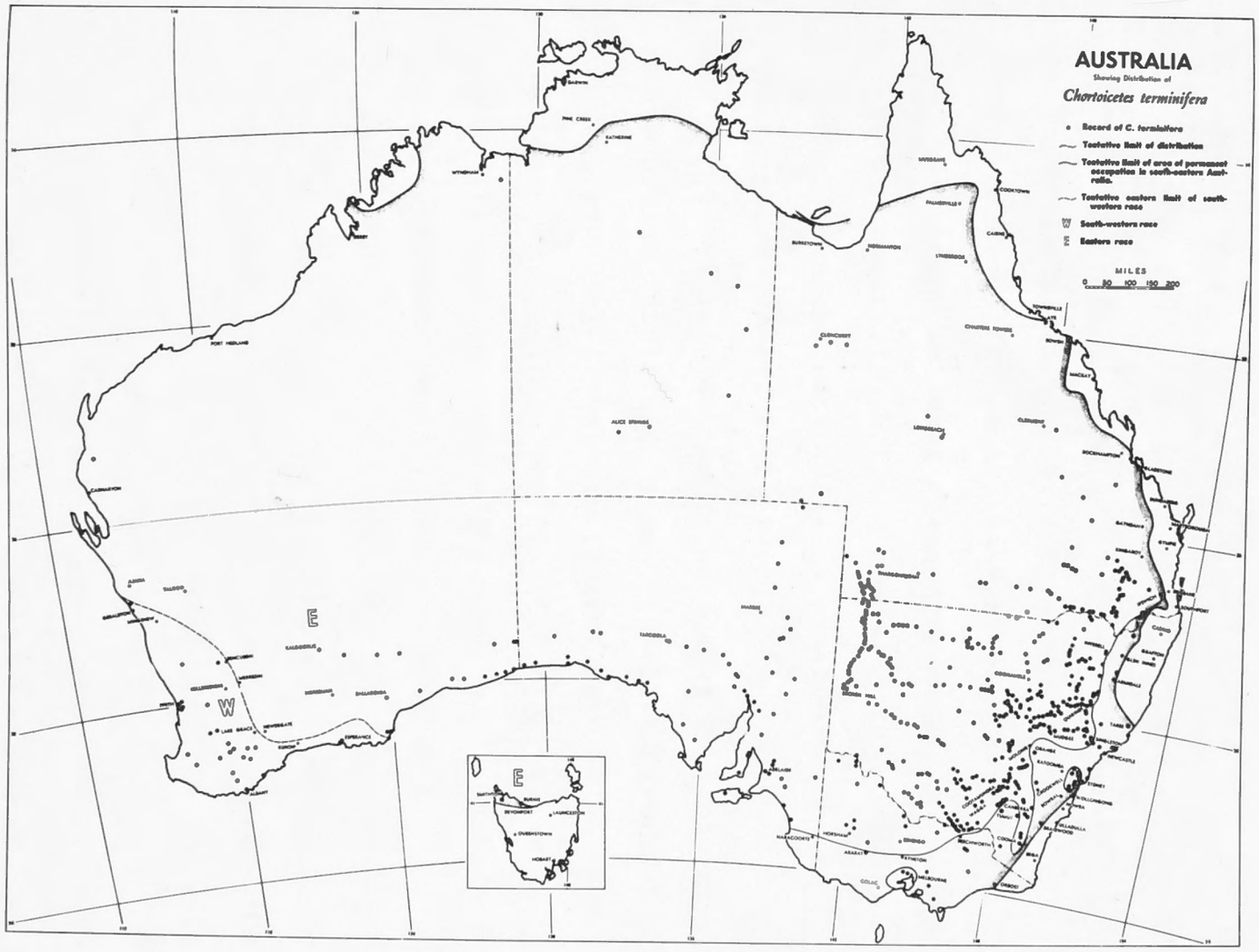
has dried. Since *Chortoicetes* is a hemimetabolous insect the nymphal stages resemble their parents in most respects except that they are wingless. Feeding begins during the first day of life, and as growth ensues, the rigid cuticle is shed at intervals which are dependent on rearing conditions. Different nymphal instars are recognised by size and by the development of wing pads (Fig. 2.3). Although there is a notable sexual dimorphism, both males and females usually reach adulthood after five moults, females rarely undergoing six. The usual case in such a dimorphism is for the female regularly to have an extra moult (Uvarov, 1966).

2.2 ECOLOGY

Chortoicetes is distributed over most of the Australian Continent (Key, 1945; Fig. 2.4). It is particularly abundant in the eastern states which have a long history of periodic outbreaks and plagues. Key mapped 20 known and 14 suspected outbreak areas, numbered in order of importance. The separation of the areas was somewhat arbitrary being based largely on climate and soil type characteristics. These areas are predominantly confined to cleared or naturally treeless country, usually very flat, in which there is a mosaic distribution of heavy self-mulching soils and lighter compact alluvial soils. Moreover, Key envisaged that the major 'outbreak areas' were situated within a climatic zone particularly favourable in its temperature and moisture characteristics for locust survival and breeding.

FIGURE 2.4

Map showing the distribution of *Chortoicetes* in Australia (after Key, 1954)


AUSTRALIA
Showing Distribution of
Chortoicetes terminifera
Record of C. terminifera
Tentative limit of distribution
Tentative limit of area of permanent occupation in south-eastern Australia.
Tentative eastern limit of south-western race
W South-western race
E Eastern race
MILES
0 50 100 150 200
E
W
E

Undoubtedly, some swarms have developed in these areas, particularly in New South Wales, and the biological and ecological studies of *Chortoicetes* have been rather confined to such areas in the past (Key, 1938, 1942, 1943, 1945; Clark, L.R., 1947, 1948, 1950; Clark, 1965, 1969). Extensive clearing of grazing lands in central N.S.W. late last century led to infestation and subsequent breeding of *Chortoicetes* in these areas (Clark, L.R., 1948, 1960). Outbreaks in these areas are usually localised and tend to be relatively short in duration rarely persisting for longer than one year - i.e. a maximum of three swarming generations (Clark, 1972).

It is interesting to note that earlier workers such as Olliff (1891) and Gurney (1925) attributed the permanent breeding grounds to the dry western parts of N.S.W. west of longitude 146°E. Albeit, it has since become clear that the origin and process of swarm formation is an intrinsically more dynamic system than that envisaged by Key (l.c.). Wardhaugh (1973) provides a detailed account of the climatic characteristics of the regions in which the main breeding areas of *Chortoicetes* are found. He deals mainly with a description, and the influence of seasonal variations of temperature and rainfall, the parameters which appear to be of most biological significance. Clark (1972, 1973a,b,c) adopted a new approach to the problem. He distinguished between outbreaks and plagues considering the role of locust abundance and fluctation of numbers in each case.

Outbreaks by definition are confined to isolated regions of population build-up which result from local breeding. These upsurges in locust numbers occur very frequently, particularly in the central west of N.S.W. between the Bogan and Macquarie Rivers (Casimir, 1962; Magor, 1970) and may ultimately lead to the development of migrating swarms (Key, l.c.). In a seven year study of the abundance of *Chortoicetes* in part of the Bogan/Macquarie Outbreak Area Clark (1973c) found that *Chortoicetes* maintained a high rate of reproduction. Its 'net' reproduction i.e. the number of surviving offspring per parent was also sufficient to maintain a relatively high average level of adult numbers in the region. Moreover, the range of fluctuation in numbers of adults was narrow. The number of adults per generation was found to be directly related to the rainfall during the three weeks following hatching. Prediction of numbers can be made by the linear regression of adult numbers on rainfall. Clark (l.c.) suggests that rain immediately after hatching favours the survival of nymphs by influencing the growth of food plants. Ecological factors and locust behaviour which cause departures of the observed from the expected numbers are also discussed.

Considering the Bogan/Macquarie Outbreak Area as an example, Key (1945) found that compact soils usually occupy the higher levels of the habitat where *Chortoicetes* is found. The bare patches amongst low cover provided by annual species such as *Agrostis avenacea* and *Eragrostis*

cilianensis (which grow rapidly following small falls of rain) constitute Key's (l.c.) 'oviposition habitat'. A 'food shelter' habitat is provided by the spaced tussocky perennial grasses which grow on the less well-drained self-mulching soils. Shelter against adverse weather conditions is provided by the tussocks, of *Chloris acicularis*, *Eragrostis serifolia* and *Themeda australis*. Perennial species (predominantly *Atriplex apinebractum*, *Chloris truncata*, *Stipa trefoils* and *Stipa variabilis*) on the heavier soil type have a longer vegetative phase than the annuals. Other perennial species such as *Sporobolus caroli* and *Stipa aristiglumis* may also occur. Although a wide range of plant species are available following adequate rainfall, quantitative gut analyses of *Chortoicetes* indicate that only a few species are selected as the principal food source (Lewis, unpublished). Approximately 50 to 80 per cent of total food intake is made up of the grasses *Chloris truncata* and *Eragrostis cilianensis*, supplemented by legumes such as *Medicago* spp. Bernays and Chapman (1973) found that *Hordeum leporinum* was also prominent in the diet of the nymphal stages. The germination and persistence of the annual species during spring and early summer is also influenced by rainfall. When dry conditions prevail, nymphs often continue to select the same preferred food plants as above (Lewis, unpublished) although they rarely eat a full meal (Bernays and Chapman l.c.). Moreover, *Chortoicetes* nymphs are very fastidious in their dietary requirements (as are *Phaulacridium vittatum* - Clark, 1967) and they will not feed to any great extent on plants with hard cuticles (such as the perennial *Cynodon dactylon*) which survive under dry conditions (Bernays and

Chapman, l.c.). The same authors concluded that a high proportion of nymphs died of dessication under dry summer conditions. Such high mortality has been considered by Clark (1973c) to counteract the relatively high adult fecundity thus reducing the expected amplitude of fluctuation in adult numbers in the Bogan/Macquarie area.

Outbreaks, then, may develop in many regions and are therefore very extensive and widely distributed (Clark, 1972). On the other hand, plagues are generated when swarms develop simultaneously or in succession in a number of outbreak areas.

Plagues are much less frequent than outbreaks and are usually only initiated in the more remote areas of western N.S.W. and Queensland. They persist for much longer time periods (measured in years rather than generations) causing considerable economic damage (Casimir, 1962; Magor, 1970, Bullen, unpublished). Itinerant breeding and mass migration are a feature of plague periods and control measures, which may reduce locust numbers in local areas, are usually not effective in preventing the continuation of a plague. The plague dynamics of *Chortoicetes* have been considered in detail by Clark (1972, 1973b) and a synopsis of his findings is presented below. Historical accounts of outbreaks and plague periods may be found in Clark, L.R. (1953), Casimir (1962) and Magor (1970).

Upsurges in numbers are triggered off by a succession of exceptionally heavy widespread rains. The initial increase in locust numbers leading to an outbreak may not be substantial, it may only involve a slight decrease in

nymphal mortality. Maturation of the resultant adults is then synchronised by the weather and locust behaviour results in concentrated oviposition leading to hopper bands in the following generation. Nymphal survival is enhanced by the foraging behaviour of hopper bands which differs from that of solitary nymphs and at fledging, concentrations of bands frequently give rise to flying swarms.

The degree of cohesion and displacement of swarms varies widely - it is not directly dependent on density (Clark, 1972). Flying swarms of *Chortoicetes* have been encountered at heights of 3,000 to 4,000 feet and above (Roffey, 1972). However, in most instances swarms have the appearance of stratiform streams and fly relatively close to the ground; i.e. at heights less than 200 feet (Clark, 1972). Accordingly in central western N.S.W. displacement directions are apparently determined by topography and swarms repeatedly move along the same routeways or migratory paths (Magor, 1970). Similar routeways have not been recognised in western N.S.W. or Queensland. Major swarm displacement is often intermittent and depends on the occurrence of sequences of particular synoptic patterns, e.g. the southerly movement of swarms which invade the more closely settled regions of N.S.W. and Victoria occur during short periods of a few days, under the influence of prevailing northerly winds (Clark, 1969, 1972; Casimir, in prep.). Clark (1973b) has shown that down-wind flight during periods of disturbed weather, when convergent winds and rainfall are probable, enables *Chortoicetes* to find suitable breeding grounds.

Displacement distances of such swarms have been recorded as exceeding 560 Kilometres (Clark, 1972). Follow-up rains are usually necessary to provide food for the hatchlings. The success and duration of locust plagues is thus due to the adaptive opportunistic breeding in habitats which are usually unsuitable.

Factors which lead to the termination of upsurges are not altogether clear although a change to dry conditions is usually associated with a decline in numbers. Once the decline is initiated there is often a spectacular increase in parasitic attack (Clark, L.R., 1953; Casimir, 1962; Hogan, 1965) and surviving locusts rapidly become dispersed.

This process of upsurge and decline in locust numbers has an analogue in a study by Ford and Ford (1930) who reported an association between variability and fluctuation of numbers in the butterfly *Melitaea aurinia*. No evidence of such a correlation has been found in *Chortoicetes* although several populations were collected in circumstances similar to that described by Ford and Ford, i.e. populations where numbers had been low and had subsequently built up into an appreciable population size (q.v. 5.4).

2.3 LIFE HISTORY UNDER NATURAL CONDITIONS

The initial description of the life history of *Chortoicetes* was by Swan (1956) whilst L.R. Clark (1949) was the first to report quantitative data on the life cycle at the population level. Byrne (1962) collected information on generation time and developmental rates for nymphal

stadia from six populations in the Bogan/Macquarie Region of New South Wales. He found that hatching continued for about three weeks after it began. Five weeks later the population consisted almost entirely of adults, the second generation emerging approximately eleven weeks after the first.

Nymphal development is not synchronous, males develop slightly faster than females and are sexually mature within a few days after their final moult. Sexual maturation in the female, measured in terms of initial oocyte development, is dependent on rain (Clark, 1965). Falls of rain which are effective in producing plant growth result in maturation. The stimuli which trigger off the endocrine changes leading to oocyte development are unknown, but once development begins females will respond to males and mate (Clark, 1972). Clark (1965) found that the period required for maturation after rainfall, was approximately 12 days, the sequence of successive layings being of the same duration. He estimated that the average number of egg pods per female in the field varied from less than one to a maximum of three. Field estimates of the mean number of eggs per pod ranged from 32.2 to 38.0 (Clark, L.R., 1947; Clark, 1965) in swarming populations to a mean of 53.11 (number of eggs present in ovarioles) for females of low density populations (Clark, 1972).

Oviposition was found to occur around the edges of bare compact soil (Key, 1942; Clark, L.R., 1947) and is largely dependent on soil moisture content (Clark, 1965)

although laying has frequently been observed under dry conditions (Wardhaugh, 1973). The distribution of egg pods is also strongly influenced by the occurrence of cracks in the soil surface (Clark, 1965). Male locusts settle in the vegetation surrounding oviposition sites and stridulate vigorously. This sound probably guides gravid females to the oviposition site. It also enables further mating directly after the female has laid her eggs. The following description of female behaviour during oviposition has been extracted from Wardhaugh (1973). When preparing to lay the female locust arches its back and forces its ovipositor into the ground with a series of jerks that coincide with the alternate opening and closing of the paired dorsal and ventral ovipositor valves. Since the abdominal intersegmental membranes are folded and elastic, the female locust is capable of penetrating to depths of 8-10 cm. When digging is completed the eggs are exuded along with a proteinaceous froth (Lees, 1966) secreted by the accessory glands. This froth not only binds the eggs together but permeates the surrounding soil and forms the walls of the egg clutch. As oviposition proceeds, the female slowly retracts her abdomen depositing the eggs in a series of layered batches, three or four eggs at a time. When egg laying is completed the remainder of the drill hole is plugged with froth; loose debris is then scraped over the froth plug and tamped down to form a detachable cap.

Two generations are usually completed in the one season (i.e. from early spring - September, to late autumn - May), the occurrence of each generation being regulated by rainfall (Clark, 1965; Magor, 1970). Three consecutive generations were noted in the central west in 1938-39, 1939-40, 1968-69 and 1972-73 (Magor, 1970; Wardhaugh, 1973) in a period of 36 years. In every case the occurrence of three generations marked the beginning of a major plague period. Temperature appears to be a limiting factor only in the cooler south western tableland regions where one generation per year is the case, e.g. Canberra, A.C.T. Eggs which are laid during autumn commence hatching in spring after the onset of warm weather. A further complicating feature of the population dynamics of *Chortoicetes* is the occurrence of a previously undetected egg diapause. The reader is referred to Wardhaugh (1973) for an explanation of the complex mechanism governing the embryonic egg diapause and subsequent egg development. It also accounts for the limited partial hatch, noted by other authors (Key, 1938; Clark, L.R., 1947; Clark, 1965) that occurs after the autumn egg laying. The resultant nymphs from this 'non-diapause' fraction of eggs over-winter as second and third instars. The arrest and subsequent development of these stages is also probably controlled by photoperiod and temperature, two of the principal components of the embryonic diapause.

Studies of the African migratory locust *Locusta migratoria migratorioides*, by Uvarov (1921) led to his

theory of phase transformation. He postulated that the 'grasshopper' or solitary phase of the migratory locust changed into the gregarious phase, through a series of intermediate stages known as transiens. The theory was placed on a firm basis by Faure (1932) who demonstrated phase transformation in the other principal species of African locusts; *viz.* *Locustana pardalina* (the brown locust); *Schistocerca gregaria* (the desert locust); and *Nomadacris septemfasciata*. However, the phase concept and in particular the validity of biometrical data as an indication of phase, has since been seriously questioned (Key, 1950; Kennedy, 1956; Ellis, 1970). Indeed the changes in colour and biometrical characters which Faure (1932) claimed were so highly correlated with population size that they could be used to determine the phase status of different populations do not occur in *Chortoicetes* (Hawke, 1970).

Two distinct races of the Australian Plague Locust are known. The first is the eastern race which occurs over most of Australia. This race undergoes periodic gregarisation and often migrates in flying swarms. Previous pages of this chapter refer to this race. The second race is known as the south-western or western race. It occupies a narrow region in Western Australia to the south and west of a line starting on the coast about Northampton and running through Morawa, Bencubbin, Merredin, Lake Grace and Ravensthorpe reaching the coast again near Point Malcolm (Key, 1954; Fig. 2.4). Swarming by this race is very rare and the small outbreaks are of a purely

local nature (Key, 1938). In fact very little is known about the ecology and biology of the western race. It should be noted that no evidence of incipient speciation has been found. Mating between the races in both directions occurs freely and there was no trace of hybrid infertility or differential viability in laboratory controlled matings (q.v. 3.3c).

2.4 LABORATORY REARING TECHNIQUES

The initial techniques for rearing laboratory cultures of *Chortoicetes* were developed by Byrne (1962). These methods have subsequently been modified by the author and Wardhaugh (1973). In the present study, locusts were maintained in six rooms which could be controlled for photoperiod and temperature. Each room was 3m. in length, 1.2m. wide and 2.5m high (Fig. 2.5). The available bench space allowed for 7 cages (of type A) per room. The temperature regimes usually employed were Night (4 a.m.-8 a.m.) - 33°C ± 1°C, and Day (10 a.m. - 10 p.m.) - 27°C ± 1°C. Between these times the temperature was rising or falling from one limit to the other. A full description of the methods of temperature control and illumination is contained in Wardhaugh (1973). Accurate control of humidity was not possible. Readings from a Lambrecht paper thermohygrograph revealed that room humidity varied from fourteen to seventeen per cent. Cage humidities were somewhat higher, the relative humidity varying from 35 per cent in the middle of wet food to 31 per cent at the edge of the food source. Locusts usually eat from the edge of the food source towards the centre, so the latter reading is probably

FIGURE 2.5

Locust Breeding Room

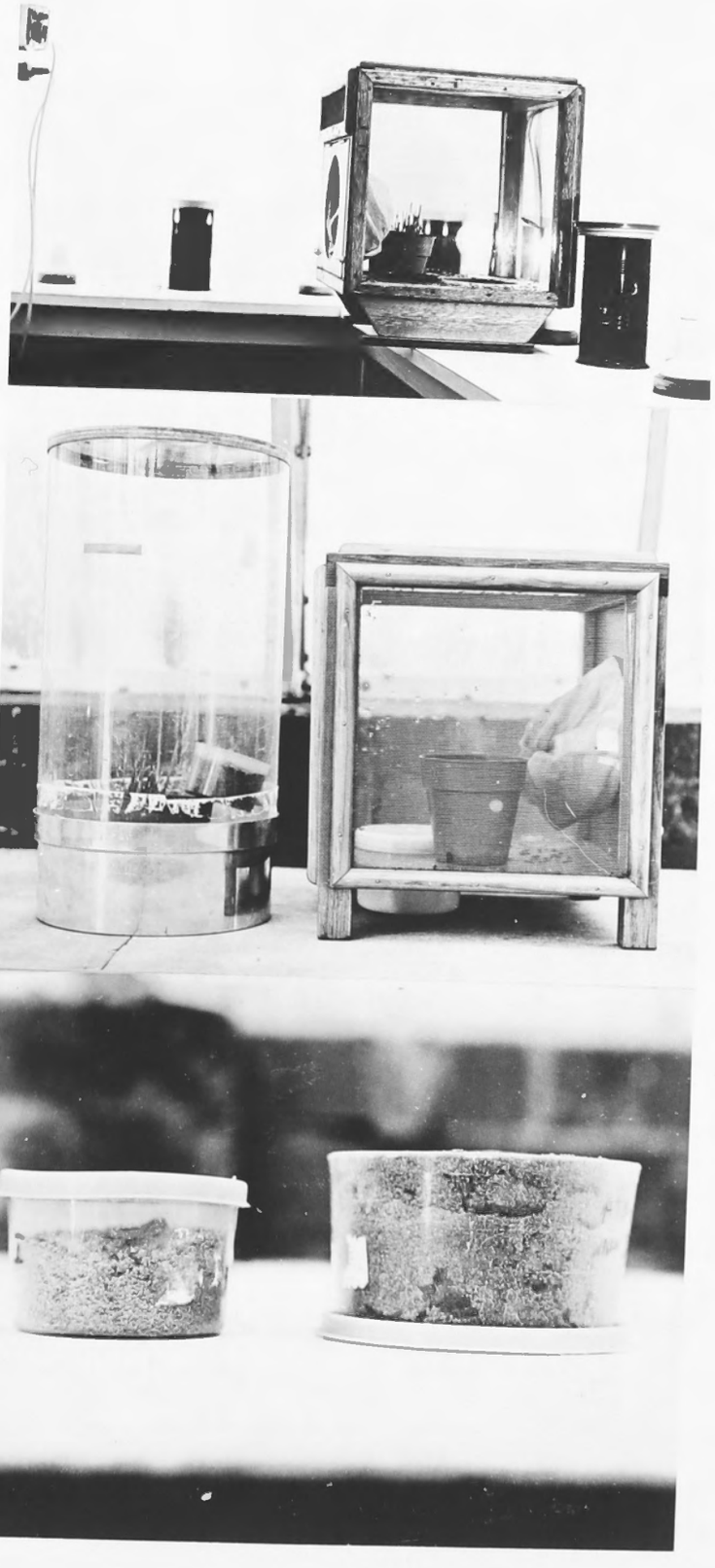
FIGURE 2.6

Cage Types Usually Employed for Breeding and Experiments

Cage Type B - left; Cage Type A - right.

FIGURE 2.7

Container Types Used for Collecting and Incubating Egg Pods



more relevant to current experimental conditions.

Two cage types were commonly employed. The main type, cage A (Fig. 2.6) was a cube of mesh with a side of 25 cm and volume 15.6 litres. All matings were carried out in these cages. Cages were placed in a standard position with their bases against a forty watt clear light globe, thus creating a temperature gradient within the cage (Fig. 2.5). Individual locusts could vary their body temperature by moving about the cage and changing their orientation to the heat radiated from the light globe. Females matured readily under the laboratory conditions employed and egg pods were collected in perspex containers (height 5cm, diameter 10.5 cm; Fig. 2.7) filled with sterilised damp soil(moisture content about 12 per cent). These containers fitted flush with the cage floor and were changed at varying intervals depending on the nature of the experiment. Egg pods were removed by washing the surrounding soil away with a water bottle. Individual pods were then placed in smaller containers (height 5 cm, diameter 7.5 cm; Fig. 2.7) of fresh sterilised soil and incubated at 33^{o}C for approximately two weeks when hatching usually occurred. If hatching did not occur after 20 days, the eggs were examined and diapause eggs were transferred to 15.5^{o}C for 40 days to break the diapause. After this period, eggs were transferred back to 33^{o}C where hatching occurred about a week later.

Cage type B (Fig. 2.6), a celluloid cylindrical cage of height 40 cm and diameter 22.5 cm (15.7 litres)

was used intermittently for rearing full-sib families to late first instar at which time sex and colour pattern were recorded. At this stage all locusts were transferred to cages of type A. Carbon dioxide anaesthetic was occasionally used when nymphal numbers, sex and colour pattern were recorded. This process was avoided where possible and general handling of the insects was kept at a minimum. The food source consisted primarily of wheat, supplemented in first instar by a dry mixture of bran, casein and dehydrated yeast (4:2:1 parts by volume). Fresh food was supplied as necessary, locusts having access to food *ad libitum*. Faeces and dried food were removed from the cages daily and rooms were vacuumed periodically. Cleanliness was essential as locusts are susceptible to pathogenic diseases which result in heavy mortality. *Serratia marcescens* and *Melamoebae locustae* are common pathogens of unhygienic cultures. Soil sterilisation prevented fungal attack of locust eggs.

2.5 LIFE HISTORY UNDER LABORATORY CONDITIONS

Both races, as well as hybrids, have been bred in the laboratory, although results pertaining to the western race are somewhat limited as yet. As with other locust species, temperature and density directly affect nymphal and adult rates of development. At 26°C the nymphal period in *Chortoicetes* averages 47 days (Hawke, 1970) compared with 34 and 65 days for *Locusta* (Hamilton, 1950) and *Schistocerca* (Dudley, 1961). At the higher temperature

of 33°C, nymphal development in *Chortoicetes* is completed in about 27 days. This compares favourably with the 27 days for *Locusta* (Hamilton , 1950) and 28 days for *Schistocerca* (Dudley, 1961) at the same temperature. These figures refer to hoppers reared under crowded conditions at constant temperatures. However it should be noted that *Chortoicetes* cultures are somewhat difficult to maintain under constant temperatures. This is especially the case when insects are reared at ambient temperatures of 26°C or less, where a high level of nymphal mortality and reduced adult fecundity result. Because of this and in order to simulate natural environmental conditions more closely, fluctuating temperatures were employed in these experiments. Additionally, mass rearing techniques were employed in the nymphal stages, so it became impractical to measure accurately the duration of each instar. Albeit, it was possible to obtain information on mean fledging dates by removing the adults from each cage as they fledged. Some of the developmental characteristics of *Chortoicetes* reared under the present environmental regime (33°/27°C) are outlined in table 2.1.

The number of instars was generally found to be five, independent of whether the hoppers were reared in isolation or under crowded conditions. In other locust species it is usual for isolated locusts to undergo one extra moult (Uvarov. 1966).

Males have been observed to copulate within two days of attaining adulthood although it is not clear whether they are invariably sexually mature at this stage. Females of both races usually copulate about four days after the

TABLE 2.1

Some Developmental Characteristics of *Chortoicetes*

Reared at 33°/27°C

		Eastern Race		Western Race	
		Crowded	Isolated		
No. of egg pods laid per female	$\bar{x}$	2.62			
	Sx	2.07			
	n	175			
Days between successive egg pods	$\bar{x}$	3.79	7.59		
	Sx	0.35	1.37		
	n	119	100		
No. of eggs per pod	$\bar{x}$	22.95	27.32		
	Sx	7.42	13.19		
	n	150	100		
Egg pod laid-hatched (days at 33°C)	$\bar{x}$	13.43			
	Sx	1.86			
	n	176			
Hatched-Fledged		Male	Female	Male	Female
Crowded conditions (11 hours light)	$\bar{x}$	26.32	26.63	25.43	27.54
	Sx	1.14	1.28	1.41	1.87
	n	142	122	123	136
Fledging to copulation (days)		4			
Copulation to egg pod (days)		4			
Generation time (egg to egg-days)		48			

final moult. Males frequently indicate that they are ready to mate by stridulating,i.e. by rubbing their hind legs against the forewings to produce a characteristic sound. This has the important function (particularly in the field) of directing mature females to mature males. During courtship the male approaches the female with a jerky stilted gait and attempts to climb upon her from the side. If the female is unco-operative she raises her hind femora into a vertical position and this is usually sufficient to deter the male. If the male persists the female will actively push him away with the hind femora. After successfully mounting, the male extends his abdomen down to one side below the female's abdomen. The sub-genital plate is turned up and becomes fixed to the female's last sternum below her ovipositor. The female may again offer some resistance at this point in which case the male reacts by convulsively contracting his abdomen. Copulation then usually ensues from ten minutes or less to several hours (up to five) at a time. In the laboratory, males may be observed in copulation mounted on the female's back, or more usually displaced from the back to a position at the side of the female. In the latter case the male faces the opposite direction to the female and copulation continues, the female dragging the male behind her as she moves around the cage.

Oviposition in the laboratory is similar to the field (q.v. 2.3). Prior to egg laying the abdomen of the female swells and often extends beyond the tips of the

hind wings. After oviposition the female will mate again if a male is available. If no male is available, the female will generally use sperm from the male she last mated with (q.v. 3.5). Up to 12 egg pods from a single mating have been recorded for several females. However, it is normally only practicable to obtain 1 to 3 egg pods from each female. There also appears to be a cyclical trend in oviposition, gravid females laying egg pods every four days on average dependent on rearing conditions (q.v. Table 2.1). Parthenogenetic reproduction has been recorded for *Locusta* (Norris, 1950) and *Schistocerca* (Hunter-Jones, 1958), but no evidence of such has been found for *Chortoicetes* in the present study. On a number of occasions the author confirmed Byrne's (1962) observation that virgin females frequently oviposited two or three weeks after moulting. These egg pods contained very few eggs which were smaller than fertilised eggs and proved to be inviable.

As with other locust species mortality occurs at two critical stages of the insects' life cycle. The first occurs about 24 hours after hatching and has been termed 'perinatal mortality' whilst the second is associated with the final moult and has been termed 'fledging mortality' (Albrecht, 1972). Some idea of the overall loss of insects from late first instar to adulthood may be gained by considering data from a study on differential viability (q.v. 4.7). Of 4960 insects set up under crowded optimal conditions, 1154 fledged, i.e. there was a 76.7 per cent loss.

2.6 ADVANTAGES OF THE ORGANISM FOR GENETIC STUDIES

Chortoicetes possesses many attributes which make

it an ideal organism for genetic study. The life cycle (i.e. egg to egg) may be completed in six weeks under optimal conditions ($35^{o}/29^{o}$, 15½ hours light) allowing the study of up to eight generations per year. Except for most Dipterans and micro-organisms, this generation time compares favourably with that of other organisms used by geneticists. Rearing is relatively easy and costs are minimal after setting up.

Each female usually lays several egg-pods (a maximum of 12 from several females have been recorded). Therefore the number of progeny from a single mating may be large, as each pod contains a mean of about 20 eggs dependent on rearing conditions (Table 2.1, Appendices 3.1 - 3.17, 3.19 - 3.27). Males are polygamous, so one male may, if necessary, be mated to several females. By using different males with the same female, distinctive progenies may be obtained in successive egg pods (q.v. 3.5). Since diapause may be induced in the egg stage (q.v. 2.7) it is possible to retain stocks not in current use for long periods if necessary. Other conditions may be utilised to circumvent diapause allowing for continuous development.

Colour patterns are developed early in the first nymphal instar enabling the study of natural selection at all stages of the life cycle. Sex may be determined from late first instar onwards. Field material is also readily available for more than six months of the year and habitats

favourable for *Chortoicetes* are distributed throughout Australia enabling genetic studies at a large number of research centres.

The presence of two separate races enables evolutionary study at the intra-specific level; whilst the many closely related *Austroicetes* species enable inter-specific comparison. Cytologically, the organism possesses excellent characteristics (q.v. Ch. 5). The ecology of the eastern race is particularly well known providing an excellent opportunity for combined ecological and genetic studies. Quantitative aspects of genetics dealing especially with diapause and biometric characters await further investigation.

Additionally, *Chortoicetes* is regarded as a serious pest in pastoral areas. Any genetical study, as well as contributing to the understanding of evolution and behaviour, may be of value in the applied science of locust control.

2.7 DIAPAUSE

Seasonal effects of external conditions are among the most important ecological factors in the life of insects (Danilyevsky, 1965; Beck, 1968). They give rise to a system of special adaptations, which are expressed in the characteristic life cycle of each species. The role of these seasonal adaptations is particularly great in the temperate latitudes where climatic variations during the course of the year are very marked. One of the most

ubiquitous adaptations concerns the possession of a definite resting stage by which the insect escapes the effects of winter cold or summer drought. The biological advantage of such a state of dormancy (diapause) is clear. The inducer of diapause (usually photoperiod) is neither directly beneficial nor injurious to the organism. If it is perceived correctly, the inducer provides an accurate and invariable indication of calendar time enabling the insect to anticipate adverse seasonal conditions.

It has been shown (Lees, 1955, 1968) that diapause may be attributed to a number of factors; e.g. temperature, and the quality or quantity of food ingested. However, these factors must be regarded as somewhat imperfect indicators of season for they are intrinsically variable and their influence on the insect is more or less immediate. Alternatively, day-length possesses all the necessary attributes. It changes methodically and the response is so tailored that the insects enter the resistant stage before the onset of unfavourable seasonal conditions.

Induction of diapause may occur in any of the major stages of the insect's life history. In *Chortoicetes terminifera*, the arrest of development occurs in the egg stage. The reader is referred to Wardhaugh (1973) for a precise description of the stages of embryonic diapause arrest and development in *Chortoicetes*.

Diapause is a highly specific feature in insects, always affecting the same morphogenetic stage of development. In many insects the potentialities for diapause are not realised in each generation. Diapause is then said

to be facultative. The arrest of growth in these species is ultimately governed by the environment and can be induced or averted by the appropriate external conditions. Other species are said to have an obligate diapause which occurs in every generation regardless of the environment. *Chortoicetes* has a facultative type of diapause, the majority of eggs laid during March and April being in diapause.

Generally, diapause is regarded as a passive stage in the life cycle. However, diapause does not merely assure survival through unfavourable seasons. No less important is the fact that it often determines the constancy of life cycles and synchronisation of developmental stages with periods of the year to which they are adapted.

Andrewartha (1952) has defined diapause as a stage during which morphological growth and development is suspended or greatly retarded. The term does not imply any specific causative factor for arrested development but by definition excludes resting conditions brought about by sub-threshold temperatures etc. It is not, however, a completely inactive stage. Physiological development usually continues very slowly, following a definite direction, which ultimately results in the capacity for active development.

Reviews and general discussions of insect photoperiodism include those of Andrewartha (1952), Lees (1955, 1959 and 1968), de Wilde (1962) and Danilyevsky (1965) . These reviews deal with the phenomena controlled by photoperiod,

and the various biochemical, ecological and physiological aspects associated with it.

2.7a DIAPAUSE IN THE FIELD

Diapause in *Chortoicetes* is of particular interest since it is a significant attribute of an overall migration and life history strategy. Through variations in diapause, *Chortoicetes* is adapted to climatically different regions where a different number of seasonal generations may be produced. Such seasonal and regional variations in the degree of diapause play a major part in determining the nature of infestations and are therefore of fundamental importance in the epidemiology of the insect. It is clear that, unless the reactions to seasonal and geographical variations in day lengths are taken into account, it is impossible to fully understand the phenology, distribution and population dynamics of insects - i.e. the phenomena with which the fundamental practical tasks of entomology are concerned.

Under natural conditions the heterogeneity of diapause response extends from continuous development through various degrees of developmental retardation, to the other end of the spectrum represented by complete arrest of development. Populations of *Chortoicetes* in the central west of N.S.W. generally show just such heterogeneity. Except where winters are unusually severe, there is an overwintering population of adults and nymphs, whilst at the same time the major proportion of the spring and summer populations are in the egg diapause stage.

On the other hand, natural populations of *Chortoicetes* around Longreach (Queensland) and Canberra (A.C.T.) represent alternative ends of the spectrum. Egg pods collected from laying swarms in the Longreach area during the diapause inducing period (early March to late April) have shown no evidence of diapause and large overwintering populations of nymphs and adults are commonly encountered (Davies unpublished). Conversely, all eggs collected from the Canberra population during the diapause inducing period have been of the diapause variety (Appendix 2.1)and no overwintering nymphs have been found despite intensive searches of the habitat by the author and C.S.I.R.O. staff on a number of occasions during the early stages of winter in the years 1970 to 1973.

In *Chortoicetes* then, as in many species, it appears from table 2.2 that high temperatures tend to avert diapause while low temperatures favour the arrest of growth. Wardhaugh (1973) demonstrated in the laboratory that this was particularly the case where temperature and photoperiod acted in concert. Thus, low temperatures combined with short photoperiods have the greatest effect in inducing diapause while long days and high temperatures avert diapause.

In an endeavour to examine some of the mechanisms underlying diapause in *Chortoicetes* a number of laboratory experiments were set up. Some of these were designed specifically to investigate the genetics of the diapause phenomenon which, to date, with the exception of

TABLE 2.2

Differences in Photoperiod According to Latitude in Some Breeding Areas of *Chortoicetes*

(Information supplied by Bureau of Meteorology, Canberra)

Location and Latitude	Photoperiod	Difference	Change per day	Temperature Mean Max	Temperature Mean Min	Difference Max	Difference Min
Canberra	13 hrs 45 mins March	2 hrs 11 mins	2.15 mins	75.9	52.4	9.4	7.3
35° 13' S	11 hrs 34 mins April			66.5	45.1		
Trangie	13 hrs 41 mins March	2 hrs 2 mins	2.00 mins	85.6	60.9	11.2	9.5
32° S	11 hrs 39 mins April			74.4	51.4		
Longreach	13 hrs 24 mins March	1 hr 24 mins	1.38 mins	94.1	68.1	6.3	8.0
23° S	12 hrs 0 min April			87.8	60.1		
Perth	12 hrs 46 mins March	1 hr 54 mins	1.87 mins	82.6	62.6	7.6	5.2
32° S	10 hrs 52 mins April			75.0	57.4		

Danilyevsky (1965), have been largely ignored or scantily treated.

2.7b MATERIALS AND METHODS

The experiments described in this section were carried out using the controlled temperature and photoperiod rooms described in 2.4. Cage type A(Fig. 2.5) was used for all studies and a range of six photoperiods (15½, 14½, 13½, 12½, 11½ and 11 hours light) was employed. Where a mixture of diapause and non-diapause eggs were present care was taken to treat the two fractions separately. The diapause was broken by subjecting individual eggs to a temperature of 15.5 °C for 40 days then transferring them to an incubation temperature of 33°C where hatching occurred progressively over the ensuing week to fortnight.

A number of stocks were used and it is useful to comment briefly on the features of each.

Western Australia

The eggs used for this stock were obtained from adults collected from the area surrounding Perth in March 1972. All eggs obtained were of the diapause variety.

Moree

This stock was developed from non-diapause eggs collected in the Moree district in January 1972.

Hillston

Eggs used to initiate this stock were collected by Wardhaugh (1973) during April 1971 from a laying swarm about 20 Km north of Hillston. A mixture of diapause and non-diapause eggs were present in the collected pods.

Trangie

A laboratory stock which has been maintained for over four years at a constant 14½ hour photoperiod. Prior to the author obtaining egg pods from it in March 1971 it had been continuously "selected" for non-diapause in that only the non-diapause fraction of offspring had been used to culture succeeding generations. The stock originated from eggs laid by adults collected on the Trangie experimental farm in the summer of 1968.

2.7c RESULTS AND DISCUSSION

The initial experiment was designed to determine the critical day-length, i.e. the photoperiod required to ensure that over 50% of the population enters a state of diapause. In some species the precision of time measurement is remarkably uniform. For example, in *Spilosoma menthastri* a difference of only 30 minutes on either side of the critical photoperiod is sufficient to reverse the response in every member of the population (Danilyevsky, 1965). Other species such as *Anopheles maculipennis messeae*, measure time less acurately, the deviations exceeding ± two hours. In this case the proportion of females in diapause gradually diminished as the day length was extended from 15 to 20 hours (Vinograda 1960). Experimental data also indicate that the critical day length depends on the temperature at which the development of light sensitive changes takes place (Danilyevsky, 1965).

Insects from each population were reared throughout their lifetime at one of the six constant photoperiods. The results obtained are summarised in table 2.3 and

TABLE 2.3

Number and percentage of diapause eggs produced by different stocks reared at a range of constant photoperiods

Stock	Photoperiod (hours light) 15½	14½	13½	12½	11½	11
Trangie						
Number of egg pods	31	12	34	26	5	
Number of non-diapause eggs	664	269	530	679	131	
Number of diapause eggs	1	0	131	0	0	
Total number of eggs	665	269	661	679	131	
% diapause	0.1 %	0 %	20 %	0 %	0 %	
Moree						
Number of egg pods	22	44	20	26	60	10
Number of non-diapause eggs	694	1187	515	816	1698	255
Number of diapause eggs	1	10	62	41	40	0
Total number of eggs	695	1197	577	857	1738	255
% diapause	0.1 %	1 %	11 %	5 %	2 %	0 %
Western Australia						
Number of egg pods	14	15	17	11		
Number of non-diapause eggs	503	438	519	279		
Number of diapause eggs	0	37	70	84		
Total number of eggs	503	475	589	363		
% diapause	0 %	8 %	12 %	23 %		

presented graphically in figure 2.8.

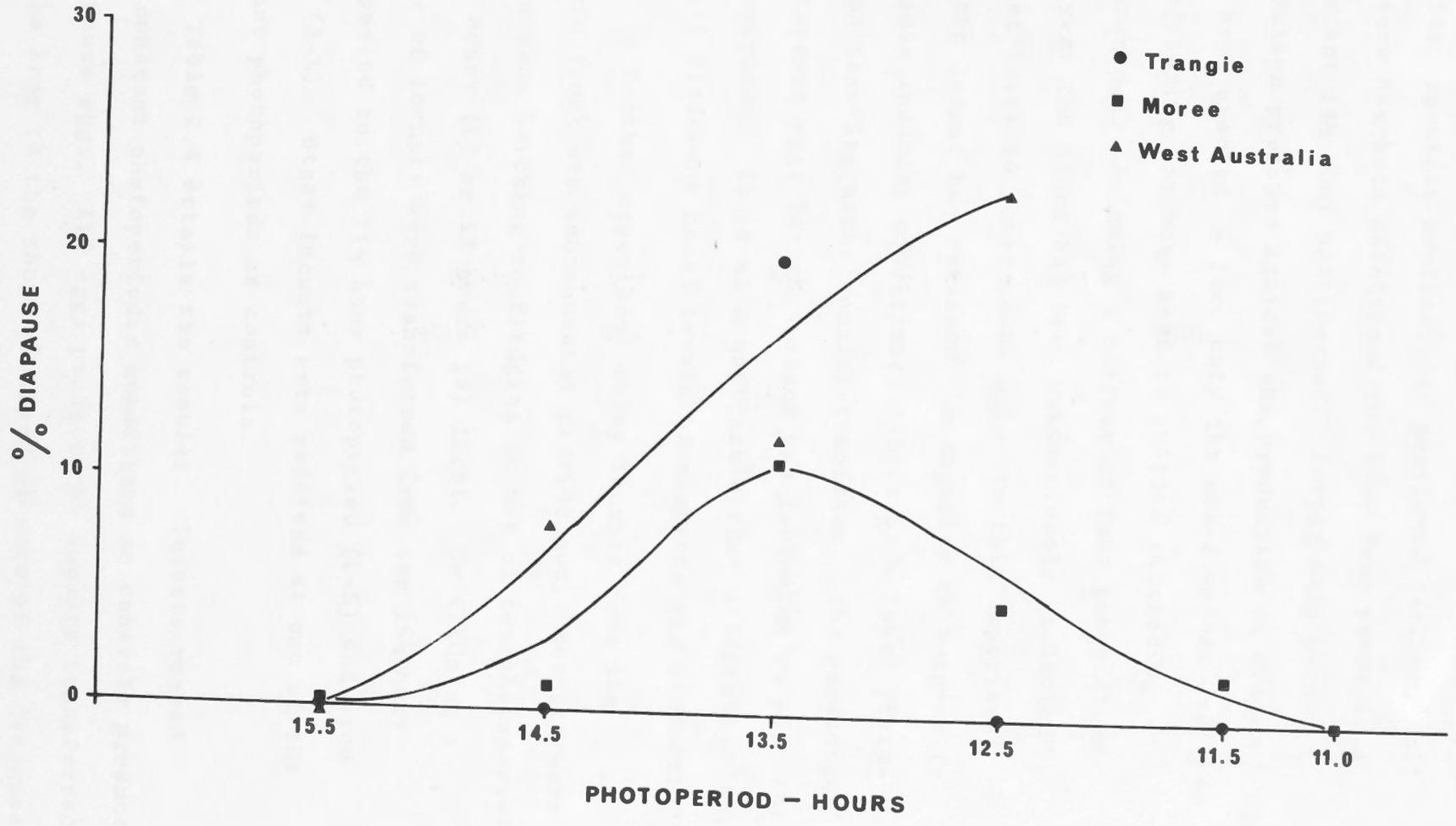
Based on a strict interpretation of the definition there is no critical photoperiod in *Chortoicetes*. However all sets of trials reveal an optimum photophase for diapause induction. A difference as small as one hour in either direction from the optimum is sufficient to bring about a marked decrease in the percentage of diapause eggs laid. At the photophases of 11 and 15½ hours diapause is virtually inhibited.

It is evident therefore, that the photoperiodic response of *Chortoicetes* is restricted to an extremely narrow range of photoperiods which fall within the range that the insect is likely to encounter under field conditions. Thus the optimal photoperiods obtained in the laboratory closely parallel the natural daylengths occurring at the onset of diapause egg laying in the field (Tables 2.2, 2.3). The Western Australian population produces an optimal response (measured in terms of the number of diapause eggs laid) at a photoperiod of 12½ hours light, whilst the eastern race populations are most sensitive to a 13½ hour photoperiod.

Variation in the critical photoperiod according to geographical location has also been demonstrated for other species, especially Lepidopterans (Danilyevsky, 1965). In *Oncopeltus fasciatus* Dingle (1974) has reported a decrease in critical photoperiod through selection which suggests that the differences observed in populations reared under different laboratory regimes are probably the result of varying selective pressures.

FIGURE 2.8

The incidence of diapause in various stocks of *Chortoicetes* reared at different constant photoperiods.


30
20
10
0
% DIAPAUSE
Trangie
Moree
West Australia
15.5
14.5
13.5
12.5
11.5
11.0
PHOTOPERIOD – HOURS

The photoperiodic response by the Trangie trial merits specific mention. As mentioned earlier, this culture has been maintained for over four years at a constant 14½ hour photoperiod. During this period a selection pressure against the production of diapause eggs has been exerted - i.e. only the non-diapause fraction of offspring has been used to culture succeeding generations. Assuming a minimum of four generations per year the stock had been unconsciously "selected" for at least 16 generations prior to this experiment. Yet the locust has retained the capacity to respond to diapause inducing conditions, albeit at a lower photoperiod than its usual rearing condition. The production of diapause eggs for this stock was inhibited at all other photoperiods. It is also noticeable that different cultures exhibit different basal levels of diapause egg production.

A further experiment using locusts from the Trangie stock was subsequently carried out. Locusts were reared from hatching to fledging at one of two photoperiods - 15½ hours (L) or 11 hours (S) light. On fledging a number of locusts were transferred from the 15½ hour photoperiod to the 11½ hour photoperiod (L-S) and vice versa (S-L). Other locusts were retained at one of the constant photoperiods as controls.

Table 2.4 details the results. Insects reared under constant photoperiodic conditions as controls produced no diapause eggs. All eggs produced by locusts transferred from the long to the short photoperiod were of the diapause

TABLE 2.4

Response by locusts transferred from long to short photoperiod (L-S) and short to long (S-L) photoperiod on fledging

Stock	Egg fraction	Treatment	No. of egg pods	No. of non-diapause eggs	No. of diapause eggs	Total eggs	% diapause
Trangie	non-diapause	L-S	13	0	344	344	100%
	non-diapause	S-L	9	232	0	232	0%
	non-diapause	L	23	505	0	505	0%
	non-diapause	S	14	314	0	314	0%
Hillston	diapause 1	L-S	217	2670	2205	4875	45%
	non-diapause 1	L-S	76	1155	638	1793	35%
	diapause 1	S-L	91	2240	0	2240	0%
	non-diapause 1	S-L	109	1968	0	1968	0%
	diapause 1	L	67	1434	61	1495	4%
	diapause 1	S	39	786	31	917	3%
	non-diapause 1	L	43	968	0	968	0%
	non-diapause 1	S	36	759	0	759	0%
	diapause 2	L-S	14	58	233	291	80%
	diapause 3	L-S	13	31	281	312	90%
	diapause 4	L-S	25	26	565	591	96%

variety and conversely all eggs produced by females transferred from the short to the long photoperiod were non-diapause. This experiment was then repeated on a larger scale using eggs of the Hillston stock treating the diapause and non-diapause egg fractions separately. As can be seen from table 2.4, the response to each treatment is in the same direction for both stocks although the magnitude of response varies. As would be expected the diapause fraction transferred L-S produced a higher proportion of diapause eggs than did the non-diapause fraction. The diapause fraction also produced some diapause eggs when reared at the constant long and short photoperiods while no diapause eggs were detected for the non-diapause fractions reared under these conditions. After diapause had been broken for the diapause fraction of the L-S Diapause 1 treatment the resultant nymphs were again reared at L until fledging when they were transferred to the S photoperiod. As table 2.4 shows for Diapause 2 the percentage of diapause eggs produced by this selection is much greater. Subsequent generations of insects subjected to the same treatment revealed an increasing proportion of diapause eggs (i.e. Diapause 3 and 4). The initial large percentage increase in diapause egg production was followed by much smaller percentage increases. A factor which complicated matters at this stage was that the period of time required to break diapause increased progressively with each generation of selection. For the first generation 95% of the diapause eggs resumed

development and subsequently hatched after 40 days at 15.5°C. The second generation required 60 days at the same temperature for a 93% hatch while 96% of the third generation resumed development after 90 days exposure to cold conditions. The diapause eggs resulting from Diapause 4 had not resumed development after 130 days at 15.5°C. Such increases in intensity of diapause have to the best of my knowledge not been reported before.

In nearly all cases studied, it has been found that insects respond to absolute levels of day-length rather than to changes. Although a response to absolute day-lengths has been obtained for *Chortoicetes* (Fig. 2.8) the greatest diapause response is elicited by a change in photoperiod rather than by the absolute day-length. Moreover, table 2.4 shows that the response is uni-directional, diapause eggs being produced only as a response to a shift from a long to a short photoperiod. This finding is consistent with field observations on the incidence of diapause where the production of diapause eggs is confined to the autumn months of March and April, i.e. to the period when day-length is rapidly declining. Few or no diapause eggs were found in egg pods obtained during spring or summer i.e. from insects which experienced increasing day-lengths or long day conditions during their period of development.

Advantage was taken of the fact that the Trangie stock produced a 100% response to L-S shift while locusts retained at L or S as controls produced no diapause eggs.

After rearing at one of the constant photoperiods some fledging females were transferred L-S but were mated with males retained at L or S. Other females were retained at L or S and mated with males transferred L-S. Matings were carried out by allowing females access to males from the appropriate experimental condition for a period of six hours each day. Although this entailed substantial handling of male locusts it does not appear likely that this would influence the results obtained.

As can be seen from table 2.5 females transferred L-S produced some non-diapause hatchlings. Similarly females retained at L or S but mated with males transferred L-S produced a number of diapause eggs. Locusts reared at L or S as controls produced no diapause eggs. It is clear from these results that the male as well as the female has an influence on the type of egg produced.

The curves obtained in figure 2.9 were obtained from stocks derived from diapause eggs collected in the Riverina district in March 1972 and are abstracted from Wardhaugh (1973). The overall difference in magnitude of response between the two generations probably indicates that the greater incidence of diapause eggs in the second generation is due to selection since eggs used for the second experiment were derived from the diapause fraction of the first generation. Table 2.6 makes this point even clearer. The percentage of diapause eggs produced in the second generation under the same constant photoperiodic conditions as the previous generation was higher for the diapause

TABLE 2.5

Effect of retention of one sex at a constant photoperiod when the other is transferred on fledging

Trangie Stock	Treatment	No. of egg pods	No. of non-diapause eggs	No.of. diapause eggs	Total eggs	% diapause
Females L-S	Males L	6	18	120	138	87%
Females L-S	Males S	8	23	131	154	85%
Females L	Males L-S	5	102	14	116	8%
Females S	Males L-S	6	140	17	157	9%
Females and Males	L	8	190	0	190	0%
Females and Males	S	8	212	0	212	0%

FIGURE 2.9

The incidence of diapause in various stocks of *Chortoicetes* reared at different constant photoperiods (after Wardhaugh, 1973).

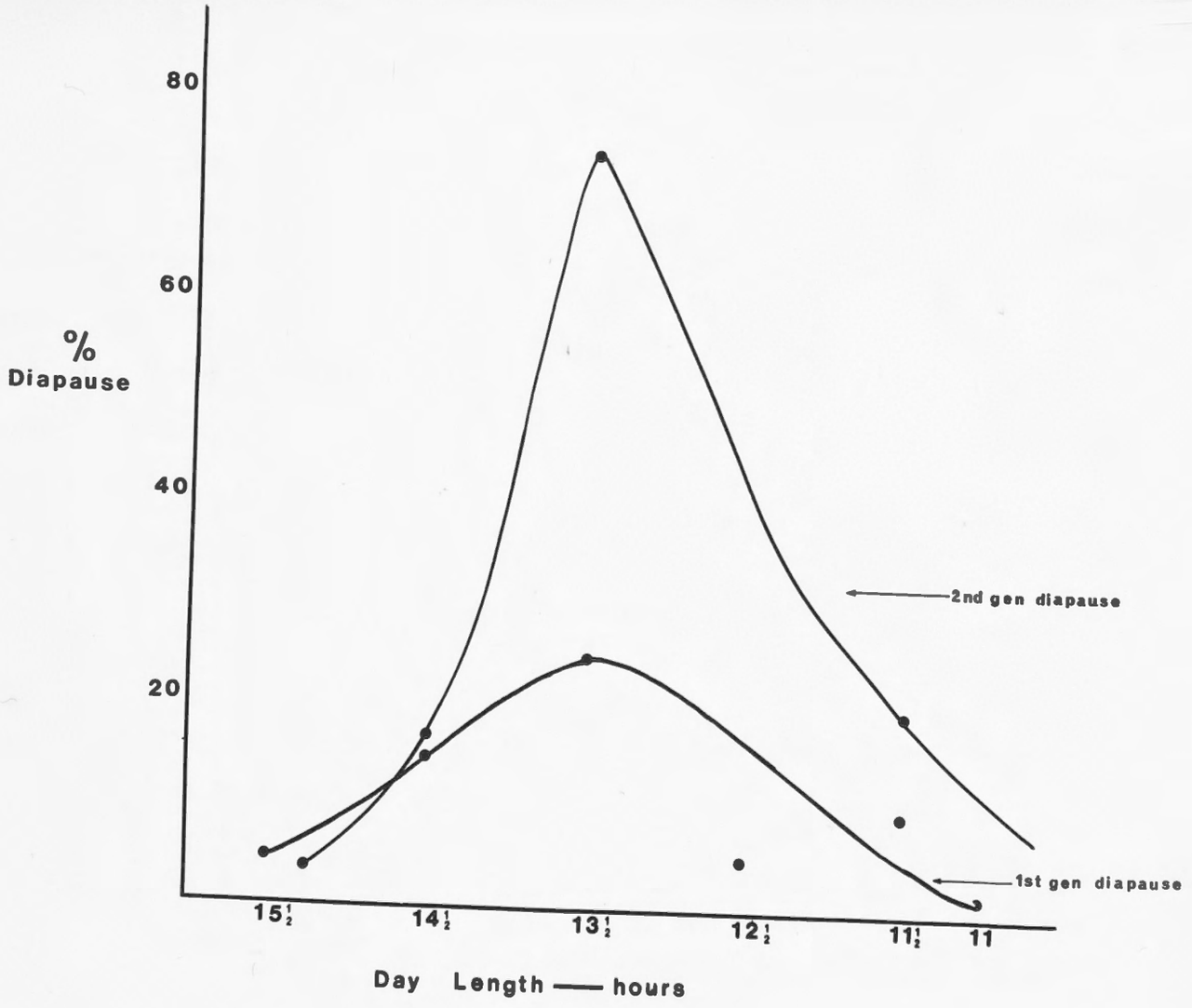

80
60
40
20
% Diapause
2nd gen diapause
1st gen diapause
15½
14½
13½
12½
11½
11
Day Length — hours

TABLE 2.6

Diapause egg production in successive generations reared at constant photoperiods.

Hillston Stock	Treatment	No. of egg pods	No. of non-diapause eggs	No. of diapause eggs	Total eggs	% diapause
Diapause 1	15½ hrs photoperiod	72	1610	85	1695	5%
Non-diapause 2	15½ hrs photoperiod	24	553	17	570	3%
Diapause 2	15½ hrs photoperiod	17	434	43	477	9%
Diapause 1	11 hrs photoperiod	49	1221	38	1259	3%
Non-diapause 2	11 hrs photoperiod	16	415	22	437	5%
Diapause 2	11 hrs photoperiod	13	314	43	357	12%

fraction than for the non-diapause fraction at both photoperiods. It is somewhat surprising that Wardhaugh's (1973) curves (Fig.2.9) intersect at the higher photoperiod although this may be due to the fact that the upper photoperiod for the second generation was 15 rather than 15½ hours.

The photoperiodic response curves in figures 2.8 and 2.9 represent the reaction of the insect population (as a whole) in terms of activity or morphological response and do not necessarily follow the intensity of response in the individual insect. A number of attempts were therefore made to account for individual differences by studying the response to directional selection at the optimal threshold for diapause induction in the eastern race. Selection experiments with other insect species have indicated the flexibility of diapause (Slifer and King, 1961; Barry and Adkisson, 1966; Pickford and Randell, 1969). Geographic variation in the critical photoperiod has been mentioned previously and crosses between different geographical races have been found to produce offspring with intermediate characteristics (Danilyevsky, 1965). On the basis of these findings it has been implied that diapause is dependent on the operation of several different genes (Lees, 1955; Barry and Adkisson, 1966; Morris and Fulton, 1970).

Quantitative genetics provide the appropriate way for analysing such polygenic characters (Falconer, 1960). The basic models of quantitative genetics assume that

individual differences in continuously varying traits are the result of both genetic and environmental differences. The total (phenotypic) variance for any trait is expressed by the equation:

$$V_P = V_A + V_N + V_E$$

where V_A is the additive genetic variance, V_N is the non-genetic variance (due to dominance and epistasis), and V_E is the environmental variance. Additive genetic variance arises from the summation of effects across all genes contributing to a trait and is the variance contributing specifically to parent-offspring resemblance (e.g. human height) as opposed to the resemblance between all members of a species (e.g. the number of fingers). The relationship between additive genetic variance and phenotypic variance is expressed by the heritability (h^2) of a trait where $h^2 = V_A/V_P$. Heritability is important because it predicts sensitivity to selection and hence the rate at which evolution can occur. Although heritability can be measured in several different ways (Falconer, 1960) the method settled on for the purpose of this experiment involved determination of the "realised heritability". Basically this meant selecting parents whose mean score, in this case number of diapause eggs per pod, differed in the desired direction from the mean of the population; the difference between the parental mean and the population mean being the selection differential (S). The mean score of the offspring of these parents is then determined, the difference between offspring mean and

population mean being the response to selection (R). Assuming that response to selection is the result of additive genetic variance, $h^2 = R/S$. Unfortunately this experiment, although attempted three times in all over a period of 12 months, was unsuccessful due to attacks of a *Coccus* (T.D. Grace, personal communication). Removal of all locusts and sterilisation with hypochlorite between the first two attempts was ineffective as subsequent experiments became re-infected and cultures died out rapidly at fledging. On reflection it appears that the stock which was used for the heritability experiment may have been particularly susceptible to attack by micro-organisms. When new stocks were introduced some four months later allied with more hygienic procedures little difficulty in maintaining disease free stocks was encountered.

A further feature of diapause in *Chortoicetes* is the presence in variable numbers (particularly at the photoperiods of 11 and 11½ hours light) of nymphs which show a pronounced delay in development during the third nymphal instar. Such nymphs resume development sporadically or when subjected to an increase in temperature and/or photoperiod resumed development within two weeks of the change (Wardhaugh, 1973). It is interesting to note that it is at this instar stage that nymphs overwinter in the field (Key, 1942). Moreover, the percentage of nymphs exhibiting retarded development was greater in insects which had hatched from non-diapause eggs (i.e.those which would normally comprise the over-

wintering population than those from diapause eggs.

At this stage of the investigation into diapause the maintenance of disease free stocks became an increasingly difficult and time consuming operation. In addition there was the problem of coping with a correlated increase in the intensity of diapause with successive generations of diapause induction and selection. Considered against the limited time period to produce results suitable for a thesis a decision was taken to turn attention to the genetic polymorphism. This decision was also influenced by the fact that a number of results obtained from matings of the different colour patterns indicated a need for further in-depth investigation because of the important implications that they seemed to be pointing to.

It is clear that the two forms of diapause described in previous pages of this chapter warrant closer attention.

CHAPTER THREE

GENETIC STUDIES OF THE COLOUR PATTERNS

3.1 INTRODUCTION

Colour pattern polymorphism is a rather common characteristic of the Insecta, particularly in the Lepidoptera (Ford, 1971) and Orthoptera (Byrne, 1967a). Unfortunately, detailed studies of such polymorphisms are available for only a few species. For example, research in the Orthopteran Order of insects has been relatively restricted to various locust species. In particular, the grouse locusts *(Tettigidae)* and their relatives have been extensively investigated by Nabours and his colleagues (1914, *et seq*). Each species of grouse locust has a number of conspicuous bright colour patterns which are dominant to a less conspicuous colour pattern referred to as the universal recessive. Most of the dominant genes are linked and in some cases no recombinants have been recovered from test matings, suggesting very close linkage or multiple allelism.

In *Paratettix texanus* thirty patterns have been found (Nabours 1914, 1917, 1929; Nabours and Foster, 1929). Twenty-three of these are extremely closely linked and are completely dominant. Three are dominant and either loosely linked with each other and with the others, or are on separate chromosomes. The three remaining colour patterns are the only ones that can be ascribed recessiveness in any way except for the normal wild-type recessive. Fifteen prominent colour pattern characteristics, all of which are located within a map distance of about eight units were found in *Apotettix eurycephalus* (Nabours 1919, 1923, 1925, 1929; Nabours and Stebbins, 1950). The genetics of

Telmatettix aztecus is simpler, there being one recessive (wild-type) and four dominant colour patterns (Nabours, 1929; Nabours and Snyder, 1928). These are either multiple alleles or are very closely linked loci as no recombinants have been recovered from test matings. *Tettigidea parvipennis pennata* has five multiple alleles (or closely linked genes), as well as one loosely linked gene which may be on a different pair of chromosomes as there is approximately 50% segregation in both sexes (Bellamy, 1917; Haldane, 1920; Nabours, 1929). In *Amblocorypha*, the green and pink colours compose a pair of Mendelian characters with the pink colour dominant (Hancock, 1916; Nabours, 1929). Additionally, there are 25 or more colour patterns in each of two *Acrydium* species, *Acrydium arenosum* and *Acrydium granulatus* (Nabours, 1929).

The other species of locust which has been studied is the Australian Plague Locust, *Chortoicetes terminifera*. The inheritance of the basic colour patterns of *Chortoicetes* has been elucidated by Byrne (1962, 1967a). True breeding stocks were established and where segregation for colour patterns occurred, ratios were approximately 1:1, 1:2:1 or 1:1:1:1. These crosses led to the postulate of a single locus with four alleles; F^a (a=albomedia F^n (n=nigrovirgata), F^r (r=rubiginosa) and F^t (t=trilineata), each allele being associated with specific phenotypic markings. Both Key (1954) and Byrne (1962) contain descriptions of the above colour patterns. Byrne's description is more accurate since it is based on knowledge gained from known matings

and takes into account the effect of various environmental modifications. For example, the colour patterns may be slightly modified, or completely masked in extreme cases, by the production of a green pigment. Production of this pigment is probably dependent on environmental factors as high humidities and an abundance of green food tend to favour its production whilst dry conditions militate against it. Other factors are also involved, since the proportion of green laboratory individuals bred under ostensibly favourable conditions is below that of field material. The proportion of green individuals also varies according to genotype, being highest for F^nF^n and lowest for F^tF^t (Byrne, 1962). Such a tetra-allelic locus gives rise to ten genotypes of which four are homozygous and six heterozygous (Fig's 3.1, 3.2). Nine of the genotypes are recognisable phenotypically in the wild, the genotypes F^aF^a and F^aF^r being indistinguishable. Among laboratory-bred siblings, the dark areas are paler in F^aF^r than in F^aF^a although subsequent environmental modification of the phenotype may obscure differentiation. Chi-square values for observed and expected numbers of progeny from 91 single pair matings indicated consistency with the multiple allelic hypothesis (Byrne, 1962).

3.2 GENETIC ANALYSIS OF THE COLOUR PATTERN LOCUS (F)

During the course of breeding experiments with the eastern and western race, 431 single egg pods were examined (355 eastern race; 96 western race) (Appendices 3.1 to 3.17). Of the 244 matings (176 eastern race;

FIGURE 3.1

Lateral View of Colour Patterns Determined by F Locus

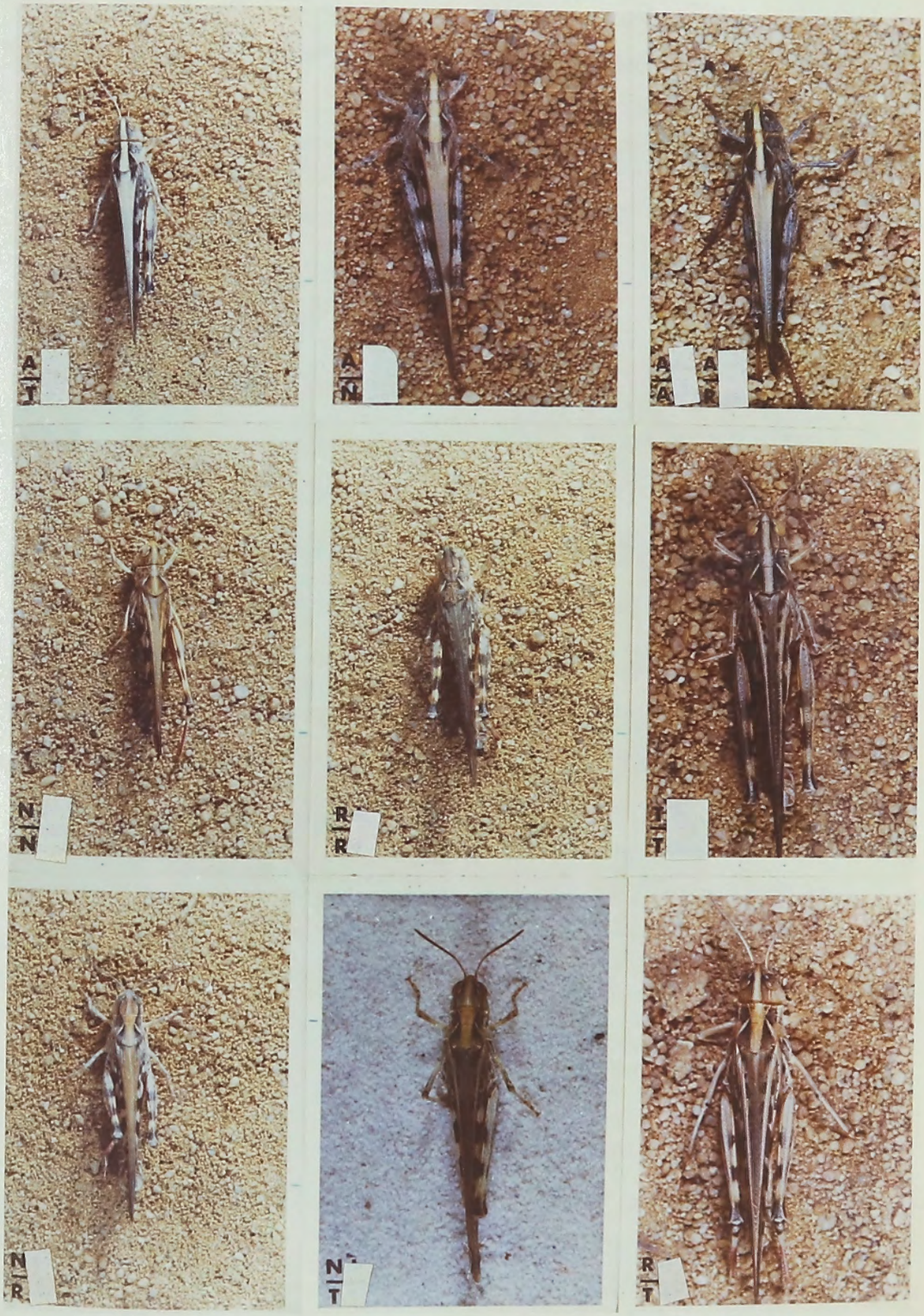
* Although it has been possible to correct most of the figures and tables which comprise this thesis, the colour plates of 3.1, 3.2 and 3.5 are labelled with the old nomenclature. The reader should ignore the + signs in figures 3.1 and 3.2 and both the + sign and the T of figure 3.5.


A T
A N
A A
A R
N N
R R
R T
N R
N T
R T

FIGURE 3.2

Dorsal view of colour patterns

determined by F locus


A/T
A/N
A/A
N/N
R/R
T
N/R
N/T
R/T

68 western race) where segregation occurred, 13 were significantly different from expected Mendelian ratios on the basis of the multiple allelic hypothesis. This is not unusual since *a priori* it is expected that 1 in 20 matings should be significantly different from expected on the basis of chance alone. Note that the expectation of some of the classes is small - the X^2 test is accepted as accurate enough if the smallest expectation is at least 1 (Snedecor and Cochran, 1967).

Byrne's (1967a) hypothesis did not exclude the possibility of closely linked loci. Thus two or more non-allelic genes may behave as multiple alleles in inheritance provided no recombination between them can be detected. The simplest model of closely linked loci that can explain the observed segregations at the 'F locus' involves three loci. In such a model rubiginosa (F^rF^r) would be represented genotypically as $\frac{ant}{ant}$. Heterozygotes would usually be in the repulsion phase such that $F^aF^t = \frac{Ant}{anT}$ (q.v. 4.5b for reasons).

It should be noted that conventional estimation of linkage distances is not possible if the model is correct as it would be impossible to distinguish between double cross-overs and cross-overs at region 1 and 2 of the three loci involved. Recombination and calculation of linkage distances would therefore be dependent on the determination of crossing-over between two loci at a time. Thus determination of the sequences A-N, A-T, N-T would usually (but not

exclusively) involve backcrossing of the appropriate phenotype to a homozygous line. For example, if "p" is the recombination percentage between A and T, the expectation of offspring in terms of "p" from a repulsion phase backcross would be :

Parents		Non-recombinants		Recombinants	
$\frac{Ant}{anT}$	X $\frac{ant}{ant}$	$\frac{Ant}{ant}$,	$\frac{anT}{ant}$	$\frac{AnT}{ant}$,	$\frac{ant}{ant}$
Relative frequency		$\frac{1-p}{2}$	$\frac{1-p}{2}$	$\frac{p}{2}$	$\frac{p}{2}$

If no recombinants were observed in "n" offspring, then the upper 95 per cent confidence limit for the recombination percentage could be calcualated by equating the first term of the expansion of $(p+q)^n$ to 0.05, i.e. $(1-p)^n = 0.05$. Table 3.1 details matings that have been used to calculate upper limits for the three regions which in turn are contained in table 3.2.

However, a series of matings resulting from a western race male collected in the field has necessitated a re-assessment of the multiple allelic hypothesis. Although there is some dominance modification and a general suppression of melanisation in specimens of the western race as compared with the eastern race, little difficulty was experienced in classifying the various colour pattern types. This male was noted to be slightly different from previous males of the same race. It appeared to have an albomedia tegmen (the albomedia gene had not previously been detected in the western race) and was test mated to an F^nF^r female. The presence of

TABLE 3.1

Colour pattern data used to estimate upper recombination limits

Mating	Appendix	Number of Matings	Total Offspring	Genotype of Offspring A/N	A/T	N/N	N/R	N/T	R/R	R/T	T/T	A/A A/R	
1. A/N x R/T		1	46		12		15	11				8	*
2. A/N x A/N	3.5	20	440	225		118						97	
3. A/T x N/N		2	23	11				12					*
4. A/T x A/T	3.7	7	119		62						28	29	
5. A/T x R/R		1	34							14		20	*
6. A/T x N/R		1	8	2				1		3		2	*
7. N/T x N/N		3	297			93		87					*
8. N/T x N/T		2	45			14		19			12		*
9. A/N x A/N	3.14	9	149	74		40						35	
10. N/T x N/T	3.9	43	1071			252		574			245		

* Data abstracted from Byrne (1962)

TABLE 3.2

Upper limits of recombination with 0.95 certainty for the regions A-N, A-T and N-T

Region	Mating	n	p%
Eastern Race			
A-N	1,2	486	1.9%
A-T	3,4,5,6	184	3.1%
N-T	7,8	342	0.93%
Western Race			
A-N	9	149	7.9%
N-T	10	1071	0.56%

recombinant types amongst the progeny would seem to indicate that more than one locus is responsible for determining the colour pattern types (Table 3.3). The simplest model which can be constructed to explain the data involves two loci (Fig. 3.3). Considering the genotypes of the parents any crossing over in the female would be indistinguishable from the parental type gametes. However, crossing over in the male would produce the following gametes A+, +T parental and AT, ++ recombinant, the latter gamete representing the recessive rubiginosa form. Thus, at least two phenotypically distinguishable classes would be expected (and are found) amongst the progeny, *viz* R/R (i.e. $\frac{++}{++}$ and N/R ($\frac{N+}{++}$). In addition a class attributable to the inheritance of A-T as a coupling phase gamete from the male parent was found. Since crossing over has only been detected between the A and T loci then the nigrovirgata gene could be considered an allelic form of either the trilineata or albomedia locus. For the purpose of simplicity it will be treated as an allele of albomedia. Symbolically, it is easier to represent the types with capitals such that $\frac{++}{++}$ = R/R (rubiginosa), $\frac{N+}{+T}$ = N/T (nigrovirgata-trilineata) etc. Where possible this convention has been adopted to make the text easier to follow.

A feature of table 3.3 is the significant discrepancy in the sex ratio, there being 15 males and 32 females ($X^2_1 = 6.14$, $p > 0.05$). No explanation for this can be offered and it must be accepted as a chance biological occurrence.

TABLE 3.3

Progeny of Cross Between A/T Male and N/R Female

(q.v. Fig. 3.3)

		Egg Pod 1		Egg Pod 2		
		MALE	FEMALE	MALE	FEMALE	TOTAL
PARENTAL	A/N	0	1	3	3	7
	A/R	1	0	2	4	7
	N/T	2	7	1	1	11
	R/T	2	6	1	1	10
RECOMBINANT	AT/N) AT/R)	0	2	0	0	2
	N/R	1	2	0	3	6
	R/R	1	0	1	2	4
	Total	7	18	8	14	47

FIGURE 3.3

Explanation of Colour Pattern Phenotypes Observed in Table 3.3 if Patterns Determined by Linked Series of Genes

$\frac{A+}{+T}$ (A/T) MALE X $\frac{N+}{++}$ (N/R) FEMALE

GAMETES		FEMALE	
MALE		N+	++
Parental	A+	A/N $\frac{N+}{A+}$	A/R $\frac{++}{A+}$
	+T	N/T $\frac{N+}{+T}$	R/T $\frac{++}{+T}$
Cross-overs	AT	N/AT $\frac{N+}{AT}$	R/AT $\frac{++}{AT}$
	++	N/R $\frac{N+}{++}$	R/R $\frac{++}{++}$

If recombination among the loci was free, equal numbers would be expected for the progeny classes. A chi-square test ($X^2_7 = 16.4893$; $p < 0.05$) showed that this was not the case indicating linkage between the A and T loci. However, due to the abnormal sex ratio and the small number of AT/N, AT/R recombinants the recombination percentage of 25.54% (12: 32 recombinant/ parental ratio) should be treated with caution.

The surviving progeny from the cross were then mated to provide a further test of the hypothesis that the colour patterns are determined by linked loci rather than multiple alleles.

The recombinants N/R and R/R were set up in known matings after they fledged . Of the three individuals available one male and one female of the R/R recombinant class produced progeny. The R/R male was mated to an N/T female who produced 5 egg pods containing 93 offspring. Segregation was as expected and no crossing over in the female was detected (Table 3.4). Similarly, the female which was mated to an A/R male produced 106 offspring there being no significant difference between the observed and expected segregations (Table 3.5). (Recombination among all but the N/T female above would not produce any recognisable recombinant types).

Only one of the five N/R females lived long enough to produce offspring although based on the model, in crossing over, the N/R type would not produce any recognisable phenotypes. This female was mated to an

TABLE 3.4

Segregation of Offspring from Mating Between an R/R Male and N/T Female

$(\frac{++}{++})$ R/R Male X $(\frac{N+}{+T})$ N/T Female

	N/R		R/T	
	Male	Female	Male	Female
Egg Pod 1	1	2	14	14
Egg Pod 2	10	3	6	3
Egg Pod 3	0	3	1	2
Egg Pod 4	6	3	4	4
Egg Pod 5	4	5	6	2
Total	21	16	31	25

$X^2_3 = 5.1935$, $p>0.1$

TABLE 3.5

Segregation of Offspring from Mating Between an R/R Female and A/R Male

$(\frac{++}{++})$ R/R Female X $(\frac{A+}{++})$ A/R Male

	A/R		R/R	
	Male	Female	Male	Female
Egg Pod 1	2	5	8	2
Egg Pod 2	9	1	8	2
Egg Pod 3	1	1	1	2
Egg Pod 4	1	5	4	13
Egg Pod 5	1	2	8	3
Egg Pod 6	3	4	1	2
Egg Pod 7	4	6	4	3
Total	21	24	34	27

$X^2_3 = 3.5093$, $p>0.3$

'N/R' male and the offspring segregated into a 1:1 ratio (Table 3.6) instead of the expected 1:2:1 ratio. The result obtained is consistent with one parent being homozygous N/N and subsequent matings showed that the 'N/R' male was in fact of this type. Thirteen matings amongst the N/N, N/R progeny classes of this mating were conducted and the 295 offspring revealed no differences from expected ratios (Appendices 3.12, 3.16).

Both females of the other recombinant class survived to adulthood and were mated to the same N/R male. Examination of their progeny (Table 3.7) indicated that they were both of the $\frac{AT}{++}$ (AT/R) type (Fig. 3.4). Moreover they were both tentatively identified as A/T before mating, there being no trace of the rubiginosa phenotype at all. This suggests that rubiginosa may act as a 'universal recessive' in some cases. The situation is undoubtedly more complex than this, since rubiginosa does not always behave as a recessive in the true sense. When it is present with the single dominants T ($\frac{+T}{++}$) and N ($\frac{N+}{++}$) the heterozygote combines the markings of the two homozygous forms as if they were co-dominant alleles. However, in the case of albomedia, the albomedia gene completely dominates such that A/R is phenotypically indistinguishable from A/A.

The progeny from these two females reveal several interesting features. In only one of the 11 individual egg pods is there segregation for all classes. All other

TABLE 3.6

Offspring from Mating Between N/R Female and Misclassified Male

$(\frac{N+}{++})$ N/R Female X 'N/R' or N/N Male?

	N/N		N/R		R/R?
	Male	Female	Male	Female	
Egg Pod 1	5	5	3	6	
Egg Pod 2	2	1	8	4	
Egg Pod 3	12	9	6	6	
Egg Pod 4	5	3	6	8	
Total	24	18	23	24	$X^2_3 = 1.1123$ $p > 0.05$

TABLE 3.7

Offspring of Mating Between AT/R Females and N/R Male

		Parental						Recombinant										Total eggs per po
		AT/N; AT/R		N/R		R/R		A/N		A/R		R/T		N/T				
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀			
Female I	Egg Pod 1							1	2	2	2	0	5					12
	Egg Pod 2	1	0					3	5	1	0	4	4					18
	Egg Pod 3	0	1	6	7	1	2	1	2	1	1	9	7	3	4			45
	Egg Pod 4					14	3	1	2	3	4							27
	Egg Pod 5							5	2	7	4	6	5					29
Total Progeny		1	1	6	7	15	5	11	13	14	11	19	21	3	4			131
Female II																		
	Egg Pod 1							4	5	5	2	5	3	3	4			31
	Egg Pod 2							5	5	8	8	12	12					50
	Egg Pod 3					8	3	0	1	1	1							14
	Egg Pod 4			1	3	3	3	2	2	1	2							17
	Egg Pod 5			2	1	3	4	2	1	3	3							19
	Egg Pod 6					6	1	1	2	1	0							11
Total Progeny				3	4	20	11	14	16	19	16	17	15	3	4			142
Grand Total		1	1	9	11	35	16	25	29	33	27	36	36	6	8			273

FIGURE 3.4

Explanation of Table 3.7

$\frac{AT}{++}$ female X $\frac{N+}{++}$ male

Gametes	Male N+	++
Female		
Parental AT	AT/N	AT/R
Parental ++	N/R	R/R
Recombinant A+	N/A	R/A
Recombinant +T	N/T	R/T

egg pods segregate for only four classes or less. Moreover, there is a striking similarity between some of the frequencies and types of colour patterns within groups of egg pods, i.e. egg pods 2 and 5 from female 1 and egg pods 3 and 6 and 4 and 5 from female 2. There appears to be no logical reason for these peculiarities unless it is due to some physiological process of reproduction. There was no possibility that the females were non-virgin as each was kept by itself in a separate cage prior to fledging and after mating. After copulation was observed, the male was permanently removed and females were retained under solitary conditions for the duration of their lifetime. Using this method, it has proved possible to obtain more than the usual two or three egg pods for each female. Under these circumstances, the female stores sperm and draws on it to fertilise each batch of eggs. Very little is known about this process and it may be the cause of the peculiar segregations. Indeed some of the results of table 3.16 were collected using a similar technique and a number of aberrant sex ratios are evident.

The departure from a 1:1 sex ratio in table 3.3 is not present in table 3.7 where 145 males and 128 females were recorded ($X_1^2 = 1.0586$; $p > 0.05$). More importantly, the number of recombinants are greater than the number of parental types and this is due, in part, to an almost complete lack of one parental type, *viz* the coupling phase double dominants. It may be that these types are very inviable (see also 4.5b). Accepting that this is the case and ignoring the coupling phase progeny because of inviability a chi-square test on the total progeny separated by colour pattern types reveals a highly significant difference

($X^2_5 = 75.0$; $p < 0.001$). This is due mainly to a deficiency in the number of N/T and N/R types while there is also an excess of the R/T type.

Other problems with the data are also evident. There is no easy explanation of the excess of recombinant over parental classes in table 3.7 (i.e. 210:63 recombinant/parental ratio). Even allowing for the suspected inviability of the coupling phase progeny the recombinant classes still outnumber the parentals. Secondly, the occurrence of a breakdown in linkage in opposite sexes in consecutive generations is puzzling.

The four parental types which arose from the origin cross (Table 3.3) were mated to each other (Appendices 3.9, 3.10, 3.14 and 3.15). Of these, some recombinant classes would have been recognised (if they had occurred) from the progeny of the A/N and N/T matings. Twelve hundred and twenty offspring from 52 egg pods were examined and no recombinants were detected.

In conclusion the data of table 3.3 and table 3.7 in particular is largely uninterpretable in terms of either hypothesis. The results certainly cannot be interpreted on the basis of a multiple allelic hypothesis. On the other hand, it is necessary to make so many assumptions and qualifications in order to interpret the results within a Mendelian framework that the results become virtually worthless as supportive evidence for a model which postulates separate loci.

3.3 ANALYSIS OF THE PORPHYRICA COLOUR PATTERN

Having considered the colour patterns determined at the F locus, there remains a further distinctive colour pattern which occurs at a relatively low frequency in most populations (Table 3.8). The colour purple is always associated with the presence of this gene which has been termed 'porphyrica'. Although the porphyrica type is genetically determined as distinct from the green colouration which is due to environmental factors it may serve as a means of camouflage by breaking up the general outline of the insect in much the same way as the green colouration. This is supported by an observation made by L.J. Chinnick (personal communication, 1970). He observed a large number of porphyrica individuals in a locality where rocky outcrops and associated lichen growth were frequent. Therefore, porphyrica which is usually maintained at a low frequency in most environments may exist in unusually high frequencies because of camouflage value and/or some associated physiological advantage.

3.3a DESCRIPTION

The initial description of the porphyrica colour pattern in *Chortoicetes* (Key, 1954) was based primarily on a study of 43 museum species in the Division of Entomology, C.S.I.R.O., Canberra. Of secondary importance was the knowledge of the porphyrica form in other closely related *Austroicetes* spp. (e.g. *A. cruciata*, *A. frater*, *A. pusilla* and *A. vulgaris*). The porphyrica type was

TABLE 3.8

Percentage of Porphyrica among Several Populations of *Chortoicetes*

Population	Number Scored			Albomedia			Porphyrica			Percentage	
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Albomedia	Porphyrica
Eastern Race											
Hay			8923			193			4	2.17%	0.05%
Boulia	1087	1428	2515	27	31	58	2	6	8	2.31%	0.32%
Lovat Creek	307	337	644	10	8	18	0	2	2	2.8%	0.31%
Western Race											
1961	65	77	142								
1971	29	37	66				0	2	2	-	3.03%
1972	45	41	86	1	0	1				1.16%	

further described by Byrne (1962). Both of these descriptions are in terms of a modified R/T pattern, as porphyrica is usually superimposed on this basic colour pattern. Indeed, it may be that porphyrica originated as a mutant form of trilineata. The following description is based on a larger number of individuals than previous and also takes into account for the first time, the appearance of the porphyrica phenotype when it is superimposed on other colour pattern types (Fig. 3.5). (Type specimens of all colour patterns dealt with in this thesis have been deposited by the author in the collection of Orthoptera, Division of Entomology, C.S.I.R.O., Canberra.) As with other colour pattern descriptions (Key, Byrne, 1.c.) the description is somewhat idealised. It does not take into account the minor differences in expression of the pattern between individuals due to the effects of phase, developmental and environmental factors, which may act to modify the phenotype.

The disk of the pronotum, knees and femoral lobes are always purple. Shoulder fasciae are usually present, but do not extend beyond the carinae onto the pronotum. That part of the pronotum beyond the posterior carinal lines and on either side of the purple stripe may also be purple, but it can be a paler yellow brown colour which is typical of the trilineata form. The pronotal lobe is also purple, although the pattern of the form that porphyrica is associated with is generally distinguishable. The sub-ocular fasciae and pale diagonal stripes are present

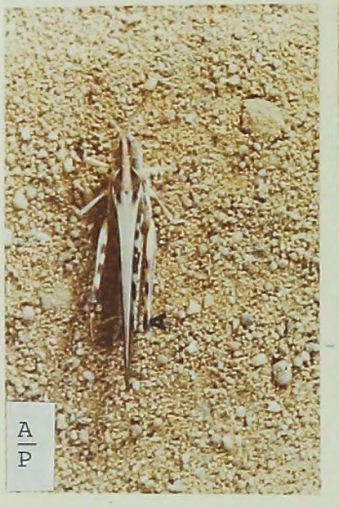
FIGURE 3.5

Porphyrica colour pattern as it appears with the other colour patterns of *Chortoicetes*


A/P


N/P


R/P


A/P


T/P


N/P


P/P


R/P


T/P


P/P

and the face is almost always entirely purple. Meso and metapleura and the anterior margin of the tegmen usually show some purple colouration.

Extensive development of green pigment is sometimes associated with the purple, especially in field material. In these individuals, green pigment is developed on that part of the pronotum beyond the posterior carinal lines on either side of the purple stripe, on the tegmen and distal portion of the hind femora.

3.3b MATERIALS AND METHODS

Porphyrica individuals were isolated from populations collected in the field. Additionally, a number of individuals were obtained from field collections made by the Locust Section of the Entomology Department, C.S.I.R.O., Canberra.

Matings between single pairs were set up using different colour pattern types as in 2.4. Egg pods were collected and after hatching, colour pattern and sex were recorded at late first instar.

3.3c RESULTS AND DISCUSSION

Porphyrica (P) individuals collected from the field were almost exclusively of the type R/P (3 N/P: over 500 R/P). Matings involving this type produced data in reasonable agreement with a 3:1 (porphyrica: non-porphyrica) ratio expected if the porphyrica gene is considered an

allele of the F locus (Table 3.9). Porphyrica acted as a complete dominant when in combination with rubiginosa and trilineata and it was only occasionally possible to identify the nigrovirgata - porphyrica form from the homozygote (q.v. Fig. 3.5). Full sibling families displayed variability in the depth and expression of the purple pigment associated with the porphyrica gene and it was not possible to separate homozygotes from heterozygotes in such matings on the basis of phenotype.

When porphyrica individuals were crossed to the albomedia type, however, a co-dominant allele situation was evident. A/P x A/P matings provided unequivocal classification of the progeny as the three genotypes were also phenotypically distinguishable (Fig. 3.6). P/P individuals from these matings subsequently provided a true-breeding homozygous porphyrica line. Table 3.9 summarises the information collected, whilst details of individual matings may be referred to in appendices 3.19 to 3.23.

In an endeavour to collect further evidence concerning the multiple allelic versus linked loci hypotheses an attempt was made to equate the data collected from porphyrica matings with the earlier data which suggested the possibility of two loci. The porphyrica gene would most easily fit this model if it were considered as an allele of the trilineata locus. Using the albomedia - porphyrica form (i.e. $\frac{A+}{+P}$) it was therefore possible to

FIGURE 3.6

Method of Establishment of True Breeding Porphyrica Stock

Gametes		Female $\frac{A}{P}$ A	Female $\frac{A}{P}$ P
Male	A	$\frac{A}{A}$	$\frac{A}{P}$
$\frac{A}{P}$	P	$\frac{A}{P}$	$\frac{P}{P}$

Expected Ratio $1\frac{A}{A} : 2\frac{A}{P} : 1\frac{P}{P}$

TABLE 3.9

Summary of Individual Matings of the Porphyrica Type

Genotype	Number of Matings	No. significantly different from Expected Mendelian Ratio	Appendix	Total Non-Porphyrica	Porphyrica
R/P	50	3	3.19	340	1117
N/P	20	1	3.20	76	261
A/P	32	0	3.22	192	218
R/P x T/P	36	0	3.21	164	487
TOTAL	138	4		772	2083

carry out extensive backcrosses to homozygous lines which enabled easy identification of progeny in an attempt to detect recombinants. Figure 3.7 diagrams the method used which also allowed for the separate estimation of crossing over in both sexes (table 3.10 for summarised details). Detection of cross overs would provide proof of the existence of at least two separate loci - alternatively, the detection of no recombinant types would lend further support to the hypothesis that the colour patterns are determined by a series of alleles at the one locus. No recombinants were detected. Table 3.11 shows details of the matings used to estimate upper limits of recombination which are contained in table 3.12.

Western Race

A further interesting finding resulted from the progeny of a single gravid western race female collected in the field in March 1970. This female, which showed no signs of the porphyrica phenotype, was classified as an R/T heterozygote. From an examination of her progeny which assorted in a 1N/R:1N/T:1R/T:1T/T ratio the male parent was deduced to be genotypically N/T (Fig. 3.8). From subsequent matings of the progeny, one particular cross resulted in porphyrica offspring (Fig. 3.9). Thus, one of the parents must have carried a recessive gene for porphyrica (Pw) and it would therefore be expected that half of the progeny would also be carrying it. The subsequent mating of an N/R male and an R/T female produced a batch of

FIGURE 3.7

Reciprocal Linkage Tests

$\frac{A+}{+P}$ male		NN ♀	RR ♀
Gametes		N+	++
Parental	A+	$\frac{N+}{A+}$	$\frac{++}{A+}$
	+P	$\frac{N+}{+P}$	$\frac{++}{+P}$
Recombinant	AP	$\frac{N+}{AP}$	$\frac{++}{AP}$
	++	$\frac{N+}{++}$	$\frac{++}{++}$

$\frac{A+}{+P}$ female		NN ♂	RR ♂
Gametes		N+	++
Parental	A+	$\frac{N+}{A+}$	$\frac{++}{A+}$
	+P	$\frac{N+}{TP}$	$\frac{++}{TP}$
Recombinant	AP	$\frac{N+}{AP}$	$\frac{++}{AP}$
	++	$\frac{N+}{++}$	$\frac{R+}{++}$

All classes recognisable genotypically and phenotypically.

TABLE 3.10

Summarised Details of Reciprocal Backcross Matings

N	Mating	A/N		N/P		
		Male	Female	Male	Female	Total
26	$\frac{N+}{N+}\sigma^{\male} \times \frac{A+}{+P}\varphi$	154	182	138	179	653
50	$\frac{A+}{+P}\sigma^{\male} \times \frac{N+}{N+}\varphi$	336	329	366	314	1345
		A/+		+/P		
		Male	Female	Male	Female	
3	$\frac{++}{++}\sigma^{\male} \times \frac{A+}{+P}\varphi$	16	12	18	17	63
16	$\frac{A+}{+P}\sigma^{\male} \times \frac{++}{++}\varphi$	86	88	64	86	324
95						2385

TABLE 3.11

Colour pattern data used to estimate upper recombination limits

Mating	Appendix	Number of Matings	Total Offspring	Genotype Offspring A/N	N/P	R/P	A/R	A/A	A/P	P/P	R/T	*	N/N
1. A/P x N/N	3.24	26	653	336	317								
2. A/P x N/N	3.25	50	1345	665	680								
3. A/P x R/R	3.26	3	63			35	28						
4. A/P x R/R	3.27	16	324			150	174						
5. A/P x A/P	3.22	32	410					112	218	80			-
6. T/P x R/P	3.21	36	651								164	487	
7. N/P x N/P	3.20	20	337									261	76

* Homozygous and heterozygous porphyrica classes could not be unequivocally separated in these matings.

TABLE 3.12

Upper limit of recombination with 0.95 certainty for the regions A-P, T-P and N-P

Region	Mating	n	p(%)
A-P	1,2,3,4,5	2795	0.12%
T-P	6	651	1.37%
N-P	7	337	3.5%

FIGURE 3.8

Segregation of the Porphyrica Gene in the Western Race of *Chortoicetes*

Parents $\varphi \quad \frac{T}{p_w} \times \frac{N}{T} \quad \sigma^{\prime}$

	T	p_w
N	$\frac{N}{T}$	$\frac{N}{R}$
T	$\frac{T}{T}$	$\frac{T}{R}$

Progeny	Observed	Expected
* NR	3	3.5
* RT	4	3.5
NT	4	3.5
TT	3	3.5

$X^2_3 = 0.292 \quad 0.5 < p < 0.9$

* Observance of these phenotypes carries the concomitant implication that the recessive p_w form appears phenotypically as rubiginosa on a multiple allelic hypothesis when it is in the heterozygous form.

FIGURE 3.9

Mating of heterozygous porphyrica from Figure 3.8

$\frac{N}{p_w}$ ♂ X $\frac{T}{p_w}$ ♀

	T	p_w
N	N/T	$\frac{N}{p_w}$
p_w	$\frac{T}{p_w}$	$\frac{p_w}{p_w}$

	Observed	Expected
N/T	4	4
* N/R	5	4
* R/T	5	4
porphyrica	2	4
	16	

$X_3^2 = 1.5 \quad p > 0.05$

* Observance of these phenotypes carries the concomitant implication that the recessive p_w form appears phenotypically as rubiginosa on a multiple allelic hypothesis when it is in the heterozygous form.

offspring which segregated 3:1 for non-prophyrica: porphyrica supporting the hypothesis (14 non-porphyrica: 2 porphyrica; $X^1_2 = 1.3333$, $p > 0.05$). Only one egg-pod was collected from this mating, although both porphyrica female offspring survived to adulthood and were successfully mated.

One was mated to a non-porphyrica male. All progeny were non-porphyrica (Fig. 3.10) again indicating the recessive nature of porphyrica in the western race. This finding was further supported by the mating of two western race porphyrica females collected in the field in 1971. These were mated to the same non-porphyrica male from the western race. Six egg pods were collected and 187 nymphs subsequently hatched, none of which showed any signs of the porphyrica phenotype. The porphyrica phenotype of both races appeared to be identical, but unfortunately a critical genetic test of allelism was not possible since these stocks were wiped out by the bacterial attack reported in 2.7c.

Eastern race porphyrica forms were then crossed with western race colour patterns. In order to distinguish between alternative forms in the two races the suffix w has been added where necessary to the western race symbols. After three generations of successive crossing to western genotypes, the eastern porphyrica gene was still maintaining its dominant character (Table 3.13, Appendix 3.28). There was no marked discrepancy between the expected and observed numbers in 16 such "backcross" matings. Porphyrica segregants usually outnumbered the non-porphyrica class indicating that

FIGURE 3.10

Porphyrica Female Mated to Non-porphyrica Male of Western Race

♀ $\frac{p_w}{p_w}$ X ♂ $\frac{N}{+}$

Gametes	p_w		Observed	Expected
N	$\frac{N}{p_w}$	(N/R)	8	10
+	$\frac{+}{p_w}$	(R/R)	12	10

$X_1^2 = 0.8 \quad p > 0.05$

the viability of the chromosome carrying the porphyrica gene was not lowered. Moreover, the dominance relationships of table 3.13 were identical with those of the eastern race.

The second western race porphyrica female was mated to a male of the eastern race which was heterozygous for porphyrica (Fig. 3.11). All 22 offspring were phenotypically porphyrica.

In interpreting these results and assuming first of all that porphyrica is an allele of the F locus then the western porphyrica form must appear phenotypically as rubiginosa unless it is homozygous, when the porphyrica phenotype is manifested (q.v. Fig. 3.9). The dominance relationships in the western race must therefore be almost the reverse of the case for the eastern porphyrica form. On page 56 the eastern race dominance relationship was derived to be:

P>R and T, and mostly N whereas A was co-dominant with P. For the western form, however, the following must apply:

N_w, T_w, $R_w > P_w$ (Fig's 3.8, 3.9).

The result of figure 3.11 leads to an important dilemma. It could mean that the western porphyrica form is dominant over the eastern nigrovirgata allele which would be paradoxical, given the complete dominance of the western nigrovirgata form over the western porphyrica form. Alternatively, if the porphyrica form is determined by a separate locus as outlined in figure 3.12, another explanation is possible. In this model the western race porphyrica form would be dominant over the wild type eastern allele (rubiginosa)

FIGURE 3.11

Western Porphyrica Female mated to Eastern Race Male Porphyrica

$\frac{p_w}{p_w}$ ♀ X $\frac{N}{P}$ ♂

Gametes N P

p_w $\frac{N}{p_w}$ $\frac{P}{p_w}$ (22 individuals - all porphyrica)

FIGURE 3.12

Explanation of Figure 3.10 in terms of two loci

$\frac{+_w p_w}{+_w p_w}$ ♀ X $\frac{N+}{+P}$ ♂

	N+	+P
$+_w p_w$	$\frac{N+}{+_w p_w}$	$\frac{+P}{+_w p_w}$

TABLE 3.13

Summarised Details of Successive Backcrosses of Eastern Porphyrica Gene into Western Race

Western $\frac{+_W}{+_W}\ \sigma$ X Eastern $\frac{+}{P}\ \varphi$

Number of Matings	R/R ♂	R/R ♀	R/P ♂	R/P ♀	Total
8	30	32	36	32	130
Expected	32.5	32.5	32.5	32.5	

$X_3^2 = 0.59$ $p > 0.7$

Generation II

Hybrid X Western $\frac{+_W}{P}\ \sigma$ X $\frac{+_W}{+_W}\ \varphi$

N	R/R ♂	R/R ♀	R/P ♂	R/P ♀	Total
1	6	7	9	10	32
Expected	8	8	8	8	

$X_3^2 = 1.26$ $p > 0.50$

Hybrid X Western $\frac{+_W}{P}\ \varphi$ X $\frac{+_W}{+_W}\ \sigma$

N	R/R ♂	R/R ♀	R/P ♂	R/P ♀	Tot
5	19	15	22	21	7
Expected	19.25	19.25	19.25	19.25	

$X_3^2 = 1.49$ $p > 0.5$

Generation III

Hybrid X Western $\frac{+_W}{P}$ X $\frac{A_W}{N_W}$

N	A/R ♂	A/R ♀	N/R ♂	N/R ♀	A/P ♂	A/P ♀	N/P ♂	N/P ♀	Total
2	5	6	7	5	2	7	7	8	47
Expected	5.875	5.875	5.875	5.875	5.875	5.875	5.875	5.875	

$X_7^2 = 4.25$ $p > 0.7$

and recessive to the wild type western allele. The eastern race nigrovirgata form would then be epistatic and not expressed in the presence of the western porphyrica form. Further examination of the western porphyrica form with eastern race rubiginosa, albomedia and trilineata types may hold the key to the dominance relationships and could also provide more information on the linked loci versus multiple allele hypotheses.

3.4 ANALYSIS OF THE BLACK MUTANT PHENOTYPE

In the process of analysing the porphyrica colour pattern, the male offspring of a full-sib mating between a porphyrica male (N/P) and non-porphyrica female (N/R) assorted in a 1:1 ratio for wild type: black mutant type. In the adult phenotype the tegmen of the mutant is entirely black in colour (Fig. 3.13) whilst the posterior end of the abdomen is also black in both sexes. The mutant can also be detected in the fourth and fifth instars as the wing pads of both sexes are black with red markings whilst the area surround the sclerotised valves of the female is also black with traces of red.

A sex linked mode of inheritance was suggested by the 1:1 segregation and initial appearance of the mutant type in only one sex (Table 3.14, Fig. 3.14). In the figure, the top half represents the female offspring, the bottom half the male. Table 3.14 presents the analysis of observed female progeny considering the gene to be a sex linked recessive, the usual method of sex determination in the Acrididae being XO male and XX female. Two egg pods from the same female were available for analysis. As apparent from the data, there was no significant deviation from expected.

FIGURE 3.13

Black Mutant Phenotype (left)

Normal Phenotype (right)

FIGURE 3.14

Initial appearance of the black mutant in *Chortoicetes terminifera*

Parents

Male		Female	
$\frac{N}{P}$	X	$\frac{N}{R}$	$\frac{X}{Xb}$

	Female Gametes			
Male Gametes	N X	R X	N Xb	R Xb
N X	N/N XX	R/N XX	N/N XXb	R/N XXb
P X	N/P XX	R/P XX	N/P XXb	R/P XXb
N	N/N X	R/N X	N/N Xb	R/N Xb
P	N/P X	R/P X	N/P Xb	R/P Xb

TABLE 3.14

Analysis of the Female Progeny, Testing 1:1:1:1 Segregation of Colour Patterns

Phenotype	Egg Pod 1	Egg Pod 2	Observed Totals	Expected
NN	0	4	4	5
NR	3	2	5	"
NP	2	3	5	"
RP	5	1	6	"

$X^2_3 = 0.4 \quad p > 0.9$

Technical difficulties prevented a fuller analysis which would have involved the individual mating of each female to a male mutant to ascertain genotypes although this was not necessary to confirm the mode of inheritance of the mutant type.

Considering the bottom half of figure 3.14, table 3.15 provides the analysis of male progeny. Although two classes were missing in the offspring, there is again no significant deviation between the observed and expected. In order to test the hypothesis further, males of type N/P Xb and N/R Xb were crossed to full sibs on the basis that if the mutant was a sex linked recessive then such matings should produce females homozygous for the black mutant in the offspring. These females were observed and a true-breeding stock was established.

This finding is quite significant since despite the intensive investigations by Nabours (1914 *et seq*) and Sansome and La Cour (1935), no spontaneously occurring sex linked genes have been previously reported in the Orthoptera. White (1951, 1973) noted that the X chromosome in Orthopteroid insects is invariably heterochromatic along its entire length and considered that it may well be relatively "inert" exept in so far as sex determination is concerned. Other black body mutants are known in the sheep blowfly, *Lucilia cuprina* (Whitten *et al*, in press) and screw worm fly, *Cochliomyia hominivorax* (La Chance *et al*, 1966) where the gene concerned is also a sex linked recessive. Like *Chortoicetes* (q.v. 5.3), the sex chromosomes are largely heterochromatic in these two species.

TABLE 3.15

Analysis of Male Progeny, Testing Segregation of Colour Pattern and Mutant Types

Genotype	Egg Pod 1	Egg Pod 2	Observed Total	Expected
N/N X	0	2	2	2.5
N/R X	2	2	4	"
N/N Xb	0	0	0	"
N/R Xb	2	2	4	"
N/P X	0	0	0	"
R/P X	2	3	5	"
N/P Xb	0	1	1	"
R/P Xb	2	2	4	"

$X^2_7 = 11.2$ $p = 0.125$

3.5 SPERM USAGE

In doing the formal genetics of *Chortoicetes*, it was possible to examine the pattern of sperm usage. These matings were particularly useful in allowing discrimination between the possibility of random mating versus selection of a mate, on the basis of colour pattern.

Observation on mating behaviour revealed that males were polygamous and females polyandrous. Generally, all males tended to share in the parentage when several were placed with one female (C of Table 3.16). In *Schistocerca gregaria* it is known that spermatozoa can fertilise eggs up to 10 weeks after the last copulation (Norris, 1954). Females of *Chortoicetes* have been recorded by the author to lay up to 12 egg pods after one period of copulation. It is of interest to know whether, after a second copulation, eggs are fertilised by sperm from the first or second copulation etc.

Using different colour pattern types it has proved possible to examine this issue. The method utilised was to make all males available to the female for two to three hours at a time. After one or more copulations was observed the males were removed and a record of the sequence of copulation by colour pattern was kept. Generally, it was found that the last male copulating with a female tended to become the parent of the predominant proportion of her offspring. In fact, it was only in A of Table 3.16 that more than one male was responsible for the progeny in a single egg pod, i.e. A/R, R/P offspring for two successive egg pods. Usually, a second copulation prevents further

TABLE 3.16

Mating Information Relating to Polygamy, Polyandry and Female Choice of Mate

A		B		C	
Female	Males	Female	Males	Female	Males
R/R	1R+/TP, 2A/A, 1R/R	R/R	4R/R, 1A/A, 2N/N 1T/T	N/N	2R/R, 1A/A

	Progeny A*			Progeny B*			Progeny C*		
	Phenotype	Male	Female	Phenotype	Male	Female	Phenotype	Male	Female
Egg Pod 1	R/R	9	18	R/R	5	1	A/N	8	7
Egg Pod 2	A/R	7	4	R/R	6	5	N/R	10	12
Egg Pod 3	A/R	7	8	N/R	11	2	N/R	7	11
Egg Pod 4	R/R	12	12	R/R	5	6	N/R	13	12
Egg Pod 5	A/R	6	9	R/R	7	9	A/N	7	9
Egg Pod 6	A/R	9	7	N/R	8	15	A/N	4	7
Egg Pod 7	R/R	10	11	R/R	8	13	N/R	17	12
Egg Pod 8	A/R	12	10	N/R	10	5	N/R	12	12
Egg Pod 9	A/R	9	11	R/R	9	8			
Egg Pod 10	A/R, R/P	5,4	4,9						
Egg Pod 11	A/R R/P	0,7	1,7						
Egg Pod 12	R/P	16	13						
	TOTAL	113	114	TOTAL	69	64		78	82

* In order to avoid adding another level of complexity to the table, the last mated male in each case can be identified by examining the phenotype of the offspring.

use of spermatozoa from an earlier mating. This is not due to competition between different colour patterns as it occurs independent of the order of males that are used for copulation. Whether the first spermatozoa are inactivated in some way, or even destroyed, by the second, or whether the second spermatophore mechanically prevents spermatozoa from the previous copulation from reaching the eggs, cannot be deduced from these observations.

CHAPTER FOUR

FITNESS COMPONENTS AND SELECTIVE FORCES ACTING ON THE COLOUR PATTERNS

4.1 INTRODUCTION

The mathematical approach to evolutionary theory was initiated by Fisher, Haldane and Wright. These authors paved the way for experimental studies on laboratory and natural populations as a test of their predictions.

In *Chortoicetes terminifera* the differences in colour pattern phenotypes are apparent in the first nymphal instar becoming more distinctive as the insect increases in size, thus providing an excellent opportunity to study the effects of natural selection. It also enables collection of information on whether or not a particular selective value remains relatively constant throughout the life-cycle or if it is only operative during a certain stage of development, e.g. adult or egg stage. The polymorphism was first studied genetically by Byrne (1962) although he did not indicate whether there was differential physiological adaptation in the laboratory and/or field.

This chapter examines the basis of the polymorphism by direct observation of the frequency of genotypes in wild populations combined with the experimental determination of differential viability/fecundity amongst the colour patterns in the laboratory.

4.2 FIELD STUDIES

Field studies of *Chortoicetes* were initiated to provide a base for the analysis of factors responsible for maintaining the colour pattern polymorphism.

Nymphal stages were collected using a hand net holding the net as close to the ground as possible whilst running though the population. It was not difficult to

collect large numbers of nymphs in this way. However, adults were much harder to collect unless they were in a dense population. Therefore, adults were collected by holding a net outside the window of a vehicle whilst driving through a population. Collecting was usually done when temperatures were high enough to support 'milling' flight, i.e. flight close to the ground. It was anticipated that flight behaviour would be independent of colour pattern and that samples would be random and representative of each population. Samples would not be random if it were established that the flying adults differed in colour pattern frequency to the population frequencies.

Eleven samples were collected representing both races of *Chortoicetes* (Table 4.1). The western race samples consist of nymphs and adults that were collected a few at a time. The 1961 population was collected in a restricted area around Perth (Byrne, 1962). The 1971/72 western race samples were collected over a wide area and the numbers (which were low) have been amalgamated . Collections from N.S.W. are widely distributed and include adults of both swarming and non-swarming populations. Since there were only two adult populations per year in the areas that have been studied, it is useful to separate the populations seasonally into spring (adults in November/ December) and autumn (adults in February/March) for purposes of comparison. The date and locality of each collection may be referred to in appendix 4.1. Three

TABLE 4.1

Population Samples of Adult Locusts Classified with Respect to Sex and Colour Pattern

Phenotype	White Cliffs Autumn 1973			White Cliffs Spring 1973			Tiboburra Spring 1973		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
R/R	330	388	718	121	53	174	84	43	127
R/T	37	239	276	46	22	68	64	24	88
N/R	32	49	81	26	17	43	18	22	40
A/A: A/R	1	12	13	6	3	9	4	1	5
A/N	0	1	1	1	0	1	1	1	2
A/T	0	3	3	4	2	6	0	1	1
N/T	0	31	31	11	11	22	3	11	14
N/N	0	12	12	8	3	11	4	6	10
T/T	2	14	16	11	1	12	3	0	3
Total	402	749	1151	234	112	346	181	109	290

Phenotype	Hay Spring 1971			Hay Autumn 1972			Boulia Autumn 1972		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
R/R	454	361	815	1826	1821	3647	111	270	381
R/T	372	261	633	289	318	607	267	405	672
N/R	497	326	823	356	382	738	331	383	714
A/A: A/R	1	4	5	36	24	60	24	21	45
A/N	2	3	5	3	7	10	0	0	0
A/T	0	0	0	4	3	7	3	10	13
N/T	55	41	96	73	71	144	18	17	35
N/N	44	49	93	101	117	218	165	158	323
T/T	14	3	17	40	38	78	168	164	332
Total	1439	1048	2487	2728	2781	5509	1087	1428	2515

	Western Race 1961			Western Race 1971/72		
	Male	Female	Total	Male	Female	Total
R/R	28	18	46	11	7	18
R/T	60	65	125	37	25	62
N/R	44	18	62	13	18	31
A/A:A/R	0	0	0	0	0	0
A/N	0	0	0	0	0	0
A/T	0	0	0	0	0	0
N/T	19	11	30	6	2	8
N/N	25	19	44	2	24	26
T/T	9	7	16	5	2	7
Total	185	138	323	74	78	152

	Coonamble Spring 1960			Trangie Autumn 1973			Coona Autumn 1973		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
R/R	140	149	289	582	192	774	257	146	403
R/T	181	202	383	394	109	503	145	118	263
N/R	123	82	205	169	147	316	41	30	71
A/A: A/R	10	2	12	27	10	37	9	4	13
A/N	1	2	3	0	2	2	1	0	1
A/T	8	3	11	2	4	6	1	0	1
N/T	74	62	136	4	11	15	3	4	7
N/N	32	23	55	13	33	46	5	4	9
T/T	23	28	51	23	2	25	10	1	11
Total	592	553	1145	1214	510	1724	472	307	779

samples were collected and scored by other workers competent in colour pattern identification and these have been specifically included to negate the effects of individual sampling error and allow a wider comparison chronologically and geographically. The Coonamble population represented the largest sample of Byrne (1962) whilst the Boulia population was collected in Queensland by Davies (unpublished).

Several sources of error are possible when classifying certain colour pattern types from field material. For example, difficulties may arise if collections are made from old populations because of the effect of cuticular darkening which obscures some of the slight differences between colour pattern types. All populations studied by the author were thus sampled when adults were relatively young. Environmental influences also play a part, e.g. lush green conditions lead to a marked increase in green colouration which particularly masks the nigrovirgata morphs. Where there was a high percentage of unscorable colour patterns in any population, that sample was excluded from the analysis. Albeit, it is also sometimes difficult to distinguish between R/R versus N/R and R/T versus T/T and misclassification may have occurred between these types. Such misclassification was thought to be insignificant in view of the experience gained from laboratory breeding of the different morphs. Each population was scored by the author ensuring that any bias would be constant across all samples.

4.2a ANALYSIS OF HETEROGENEITY WITH RESPECT TO SEX WITHIN AND BETWEEN POPULATIONS

The raw data for each population reveals that the genes rubiginosa, nigrovirgata and trilineata were generally frequent, whilst albomedia was rare (Tables 4.1, 4.9). A chi-square analysis testing for heterogeneity in colour pattern frequency between the sexes for each population revealed seven significant, two bordering on significant and two non-significant differences (Table 4.2). Albomedia types were amalgamated into one class for the purpose of this analysis. The chi-square value for all populations tested together for heterogeneity also revealed a signficant difference in the colour pattern frequencies between the sexes. Allowance for the disproportionate contribution to the chi-square value by the albomedia types (by disregarding them) made no difference to the probability level (Table 4.2). It may therefore be reasonably assumed that there are distinct differences in colour pattern frequences between the sexes from the one population. This could result if males and females have different genotypic selective values. If this were so, then greater differences in colour pattern frequencies between the sexes would be expected among adults than nymphs. Although it has not proved possible to collect evidence on this point, Byrne's (1962) data indicate that such is not the case. Therefore, either males and females do have different selective values or, if they are the same, the observed difference may be due to the fact that females develop at a slower rate than males.

TABLE 4.2

Analysis of Heterogeneity with Respect to Sex Within and Between Populations

1. WITHIN POPULATIONS

Population	Year	Season	X^2	D.F.	P
Hay	1972	Autumn	4.10	6	>0.6
Hay	1971	Spring	15.55	"	*
Boulia	1972	Autumn	53.74	"	***
Coonamble	1960	Spring	16.88	"	**
Trangie	1973	Autumn	126.50	"	***
Coona	1973	Autumn	11.50	"	>0.05
White Cliffs	1973	Autumn	128.39	"	***
White Cliffs	1973	Spring	7.73	"	>0.25
Tibooburra	1973	Spring	23.89	"	***
Western Race	1961		9.85	5	>0.05
Western Race	1971/ 1972		25.83	5	***

2. BETWEEN POPULATIONS

All genotypes	4410.92	60	****
Disregarding Albomedia	4339.09	50	****

The statistical convention of denoting probability levels has usually been followed in the text and tables of this thesis, i.e. $p \leq 0.05$ is marked by *, $p \leq 0.01$**, $p \leq 0.001$*** and $p \leq 0.0001$****.

4.2b ANALYSIS OF COLOUR PATTERN HETEROGENEITY BETWEEN POPULATIONS

A further analysis was carried out to determine if there were differences in the colour pattern frequencies between the population samples. Only total numbers for each colour pattern were considered and the albomedia types were accumulated into one group to prevent a disproportionate contribution to the significance level. Table 4.3 reveals significant differences between the genotypic frequencies of all populations (with two exceptions) at the 0.001 probability level. The first exception is the White Cliffs (1973 spring) versus Tibooburra (1973 spring) population which shows a significant difference at the 0.01 probability level. These two populations resulted from hatchings of the same immigrant swarm (i.e. White Cliffs, autumn, 1973) which laid in both areas (Clark, D.P., personal communication). Both populations are also significantly different from their parental population. The factors responsible for such population differences will be considered in the following pages of this chapter.

No significant difference was found between the two western race populations (Table 4.3). This result may be due to a number of factors. As mentioned earlier each sample did not consist entirely of adults, whilst the areas that the samples were collected over also differed considerably.

TABLE 4.3

Analysis of Colour Pattern Heterogeneity among Populations

Chi-Square and probability level for population comparisons (5 degrees of freedom)

	Boulia (Qld) 1972 Autumn	Trangie 1973 Autumn	Coona 1973 Autumn	White Cliffs 1973 Autumn	White Cliffs 1973 Spring	Tibooburra 1973 Spring	Western Race 1971/72
Hay 1971 Spring	*** 606.19	*** 171.23	*** 231.30	*** 408.13	*** 103.90	*** 44.64	*** 121.06
Boulia 1972 Autumn		*** 669.09	*** 597.47	*** 996.51	*** 32.35	*** 209.56	*** 37.43
Coonamble 1960 Spring		*** 266.44	*** 43.95	*** 134.28	*** 73.39	*** 30.96	*** 145.38
White Cliffs 1973 Autumn			*** 35.21		*** 42.64	*** 42.63	*** 230.15
Coona 1973 Autumn					*** 57.53	*** 31.09	*** 166.90
White Cliffs 1973 Spring						** 13.56	*** 89.35
Tibooburra 1973 Spring							*** 64.81

	Western Race 1971/72
Western Race 1961	3.60 p>0.6

Overall then, each population sample may be considered as a discrete unit, even when they are relatively close together geographically - e.g. White Cliffs and Tibooburra are 192 Kilometres apart by air.

4.3 PANMIXIS

Laboratory experiments like those discussed in 3.5 are always open to the criticism that they do not accurately reflect what happens under field conditions. Accordingly, three methods were devised for determining whether random mating among the colour pattern types occurred under field conditions.

4.3a PROGENY ANALYSIS

During the study it was possible to sample the hatchings from an egg bed where the genotypic constitution of the parents was also known. A laying swarm located near Trangie was sampled and the adult genotypic frequencies recorded. The egg bed was then 'monitored' and when hatching began, samples were taken periodically. The raw data for the population is presented in table 4.4. About 1,000 nymphs were scored for the first three instars, but after this period the nymphs became very scattered and it was only possible to collect small samples. Gene frequencies for each instar were calculated for comparison with the parent population (Table 4.5).

A Hardy-Weinberg analysis established that the first instar sample was not significantly different from expected ($X^2_5 = 4.5212$, $p>0.3$). This return to equilibrium

TABLE 4.4

Instar Samples Classified by Colour Pattern

for the Trangie 1973 Spring Population

Phenotype	Adult Parent (Trangie Autumn 1973)	Nymphal Instar I	II	III	IV	V
Male						
R/R	582	268	145	218	156	56
R/T	394	135	114	123	101	39
N/R	169	139	103	84	66	18
A/R	27	6	7	12	3	0
A/N	0	3	1	6	2	0
A/T	2	1	1	2	0	0
N/T	4	30	12	28	1	0
N/N	13	10	12	12	5	1
T/T	23	16	11	27	5	3
	1214	608	406	512	339	117
Female						
R/R	192	171	212	206	85	43
R/T	109	99	148	173	123	44
N/R	147	82	116	155	34	11
A/R	10	3	3	17	0	1
A/N	2	2	2	6	0	0
A/T	4	1	1	2	1	0
N/T	11	22	7	35	3	1
N/N	33	18	21	26	10	3
T/T	2	22	16	12	11	4
	510	420	526	632	267	107

TABLE 4.5

Gene Frequencies for each Instar Sample
for the Trangie 1973 Spring Population

	Gene			
Male Instar	R	N	T	A
Parent	.7224	.0820	.1837	.0120
I	.6711	.1579	.1628	.0082
II	.6330	.1724	.1835	.0111
III	.6396	.1387	.2021	.0195
IV	.7109	.1165	.1652	.0074
V	.7222	.0855	.1923	-
Female Instar				
Parent	.6373	.2216	.1255	.0157
I	.6262	.1690	.1976	.0071
II	.6568	.1587	.1787	.0057
III	.5989	.1962	.1851	.0198
IV	.6124	.1067	.2790	.0019
V	.6636	.0841	.2477	.0017

conditions is dependent on random mating of the parental types. Alternatively, non-random mating acting in concert with intensive selection at the gametic and/or egg stage could be responsible for the equilibrium. The precise balance required between the two opposing forces makes this latter possibility unlikely.

It was not possible to augment the first method by carrying out the other devised tests since one has to be in 'the right place at the right time' to carry out such analyses. However, the tests are included in the text for purposes of information.

4.3b ANALYSIS OF COPULATING PAIRS

The collection of mating pairs in a newly matured population may provide information on the mating strategy of *Chortoicetes*. It has been possible in the past to collect mating pairs in nets and drop them into 40% alcohol where the pairs remain fixed (Byrne, personal communication). The expected proportion of mating pairs for the colour pattern types could be estimated from the gene frequencies of the sampled population, and a chi-square test of goodness of fit would reveal any deviation from random mating.

4.3c OVIPOSITION ANALYSIS

The third method devised involved the capture of gravid females from the field and bringing them back to the laboratory. Collection of egg pods from each female and scoring of the subsequent progeny would enable the prediction of the colour pattern of the last mated male for each female. By comparing the collected information with

the known frequencies of each genotype in the male parent population a chi-square goodness of fit test would establish the presence or absence of panmixia with respect to colour pattern.

4.4. CYCLICAL SELECTION

Considering in further detail the data obtained for the progeny analysis study an interesting trend is evident. Hardy-Weinberg tests for each instar and the parental types revealed four significant and two non-significant differences (Table 4.6). The return to Hardy-Weinberg conditions in the first instar has already been explained in 4.3a. However, the alternation between conformity and non-conformity for instars 2, 3 and 4 appears to be inexplicable. It is probable that the non-significant difference of the third instar is due to a chance biological occurrence or sampling error, the chi-square value being only 0.289 short of significance. The non-agreement with Hardy-Weinberg is treated in greater detail under 4.5.

An examination of the gene frequencies was then carried out (Table 4.7). Generally, a significant increase in the frequency of the nigrovirgata gene and a corresponding decrease in the frequency of rubiginosa between the adult and nymphal samples was found (Table 4.8). Moreover, the White Cliffs autumn and spring and White Cliffs autumn/ Tibooburra spring populations revealed a similar pattern (Table 4.7). Again the nigrovirgata gene is favoured whilst

TABLE 4.6

Chi-Square Values for Hardy-Weinberg Test of the Trangie Spring Population

	Instar	X^2_5	P
Parental Estimate		23.0986	***
Instar Estimate	I	4.5212	>0.3
"	II	40.9653	***
"	III	10.7807	>0.05
"	IV	48.2134	***
"	V	12.7908	*

TABLE 4.7

Chi-square Values for Test of Gene Frequency Differences

		Trangie Adult-Nymph		White Cliffs Adult autumn- Adult spring	
		Chi-square	Probability	Chi-square	Probability
Male	Gene A	1.0611	> 0.3	6.8114	**
	Gene N	53.2787	***	12.9217	***
	Gene R	31.5912	***	51.0407	***
	Gene T	4.4512	*	27.6630	***
Female	Gene A	3.0044	> 0.05	4.4414	*
	Gene N	3.8551	*	8.8747	**
	Gene R	6.6687	**	1.5812	> 0.2
	Gene T	10.1987	**	1.0412	> 0.3

		White Cliffs Adult autumn Tibooburra Adult spring		Hay Adult Spring-Adult Autumn	
Male	Gene A	0.0026	> 0.9	0.0057	> 0.5
	Gene N	0.55	> 0.1	20.65	**
	Gene R	106.75	***	391.25	***
	Gene T	6.99	**	9.69	**
Femeal	Gene A	0.14	> 0.9	0.03	> 0.5
	Gene N	14.11	***	32.41	***
	Gene R	418.41	***	771.25	***
	Gene T	31.68	***	19.40	***

TABLE 4.8

Gene Frequencies for Successive Generations at Different Seasons

* Data taken from Table 8 of Byrne, 1967b.

Trangie	Rubiginosa		Nigrovirgata		Trilineata		Albomedia	
	Male	Female	Male	Female	Male	Female	Male	Female
Autumn Adult	0.7224	0.6373	0.0820	0.2216	0.1837	0.1255	0.0120	0.0157
Spring Nymph	0.6711	0.6262	0.1579	0.1690	0.1628	0.1976	0.0082	0.0071
White Cliffs								
Autumn Adult	0.9080	0.7183	0.0398	0.0701	0.0510	0.2010	0.0013	0.0107
Spring Adult	0.6838	0.6608	0.1154	0.1518	0.1714	0.1652	0.0235	0.0224
Tibooburra								
Spring Adult	0.7017	0.6101	0.0829	0.2110	0.2017	0.1652	0.0139	0.0138
Hay								
Spring Adult	0.6178	0.6264	0.2230	0.2233	0.1551	0.1469	0.0010	0.0033
Autumn Adult	0.7942	0.7849	0.1162	0.1248	0.0817	0.0841	0.0079	0.0061
Population A*								
Spring Nymph	0.363	0.374	0.241	0.333	0.344	0.260	0.052	0.033
Spring Adult	0.491	0.532	0.235	0.172	0.258	0.289	0.029	0.008
Summer Adult	0.518	0.549	0.189	0.137	0.277	0.306	0.016	0.008
Population B*								
Spring Nymph	0.460	0.391	0.246	0.282	0.268	0.300	0.026	0.027
Spring Adult	0.495	0.596	0.243	0.180	0.257	0.224	0.010	0.008
Summer Adult	0.591	0.551	0.134	0.225	0.259	0.217	0.016	0.007

the rubiginosa gene is selected against between the two adult populations (Table 4.8).

The relative frequency of albomedia was less than five per cent and it is therefore difficult to interpret with confidence any fluctuations that it may appear to undergo. There also appeared to be no consistent pattern for the trilineata gene.

Now, if the equilibrium is stable, counter selection of these genes at some other stage would be expected. In fact, an examination of the Hay populations reveals that in successive generations from spring (November) to late summer (February) exactly the reverse happens. This finding is consistent with that of Byrne's (1967b) report on two natural populations in the Coonamble district of N.S.W. (Table 4.8). In these populations the frequency of rubiginosa increased at the same time as the frequency of nigrovirgata decreased. Again, the frequency of albomedia fluctuated in both directions. The frequency of trilineata generally decreased in these three populations between successive adult generations.

Since these results were collected from different populations in different seasons they do not provide conclusive evidence of the existence of cyclical selection. Albeit, the consistency of the trends among different populations strongly indicates the existence of a system of cyclical selection which acts particularly for and against the rubiginosa and nigrovirgata genes in a complementary way

causing their frequencies to fluctuate seasonally. In studying such fluctuations it would be desirable to confirm the above by observing the one population over several successive generations and seasons. The itinerant breeding nature of *Chortoicetes* as described in 2.2 makes this very difficult unless the commencement of such a study was fortunate enough to co-incide with the onset of a plague period.

Since the environment in which the locust breeds varies considerably between generations and seasons, the optimum phenotype and consequently the optimum genotype may shift accordingly. Such shifts could result in selection in one direction for one or more generations or seasons followed by selection in the opposite direction for the succeeding generation(s) or season(s). Cyclical selection would obviously help to maintain genetic differences in a population in such a situation since the differences would sooner or later be advantageous. Cyclic fluctuations have also been reported for Drosophila (Dobzhansky, 1947) and more recently for vole populations (Gaines and Krebs, 1971).

4.5 MAINTENANCE OF THE COLOUR PATTERN POLYMORPHISM

Hardy and Weinberg showed that in the absence of forces that change gene frequencies in a population, the 'relative frequency of each allele remains constant from generation to generation'. The binomial distribution of autosomal genotypes is therefore approximated in a single generation of random mating and is maintained in successive generations. This law may be applied to the population samples to determine whether the observed polymorphism is in

Hardy-Weinberg equilibrium.

The gene frequencies for each population are presented in table 4.9. Expected numbers of each genotype were calculated from the male times female gene frequencies and sample sizes. A chi-square goodness of fit between the observed and expected values was then carried out. There were significant departures in 30 of the 33 sample tests (Table 4.10). All of the populations were significantly different from expected when males and females were combined.

Such highly significant departures indicate that the polymorphism is not in Hardy-Weinberg equilibrium. However, the Hardy-Weinberg law describes only the statics of Mendelian populations. If the frequencies of all genes remained constant in a population, this would represent a stage of evolutionary inertia in that population. Considering the dynamics of populations, there exist several genetic forces which disturb equilibrium conditions and alter gene frequencies in populations.

In deriving the Hardy-Weinberg law it was assumed that the population was infinitely large. Populations that consist of small numbers of breeding invidivuals are subject to random genetic drift, i.e. accidental fluctuations from generation to generation. Such a situation would be relatively rare in the eastern race of *Chortoicetes* where effective population sizes are usually upwards of the order of several thousand. It is also highly unlikely that mutation is a force to be reckoned with in the

TABLE 4.9

Gene Frequencies for Each Population

POPULATION			R	N	T	A	Pop'n size
Hay Spring	1971	male	0.6178	0.2230	0.1551	0.0010	1439
		female	0.6264	0.2233	0.1469	0.0033	1048
		mean	0.6214	0.2232	0.1534	0.0020	
Hay Autumn	1972	male	0.7942	0.1162	0.0817	0.0079	2728
		female	0.7849	0.1248	0.0841	0.0061	2781
		mean	0.7896	0.1206	0.0830	0.0070	
Boulia Autumn	1972	male	0.3883	0.3124	0.2871	0.0125	1087
		female	0.4724	0.2507	0.2661	0.0109	1428
		mean	0.4359	0.2773	0.2752	0.0115	
Coonamble Spring	1960	male	0.5017	0.2213	0.2610	0.0161	592
		female	0.5281	0.1736	0.2921	0.0064	553
		mean	0.5144	0.1983	0.2759	0.0113	
Trangie Autumn	1973	male	0.7224	0.0820	0.1837	0.0120	1214
		female	0.6373	0.2216	0.1255	0.0157	510
		mean	0.6972	0.1233	0.1665	0.0130	
Coona	1973	male	0.7511	0.0583	0.1791	0.0117	472
		female	0.7232	0.0684	0.2020	0.0066	307
		mean	0.7400	0.0623	0.1880	0.0096	
White Cliffs Autumn	1973	male	0.9080	0.0398	0.0510	0.0013	402
		female	0.7183	0.0701	0.2010	0.0107	749
		mean	0.7845	0.0595	0.1486	0.0074	
White Cliffs Spring	1973	male	0.6838	0.1154	0.1774	0.0235	234
		female	0.6608	0.1518	0.1652	0.0224	112
		mean	0.6763	0.1271	0.1734	0.0231	
Tibooburra	1973	male	0.7017	0.0829	0.2017	0.0139	181
		female	0.6101	0.2110	0.1652	0.0138	109
		mean	0.6672	0.1310	0.1879	0.0138	
Western Race	1961	male	0.4325	0.3054	0.2622	-	185
		female	0.4312	0.2428	0.3261	-	138
		mean	0.4318	0.2786	0.2895	-	
Western Race 1971/72		male	0.4865	0.1554	0.3581	-	74
		female	0.3654	0.4359	0.1988	-	78
		mean	0.4243	0.2993	0.2763	-	

TABLE 4.10

Chi-Square Values from Test for Hardy-Weinberg Equilibrium in Each Population

Population	Season	Date	Male	Female	Mean
Hay	Spring	1971	122.4160***	64.0714***	175.3027***
Hay	Autumn	1972	203.1494***	224.6506***	419.4373***
Boulia	Autumn	1972	369.8954***	249.9889***	558.5624***
Coonamble	Spring	1960	24.1891**	30.1591***	34.6657***
Trangie	Autumn	1973	172.1103***	105.0234***	143.6105***
Coona	Autumn	1973	14.6449	34.1588***	39.3777***
White Cliffs	Autumn	1973	53.3198***	224.1573***	97.1746***
White Cliffs	Spring	1973	16.1173	11.1771	19.2801*
Tibooburra	Spring	1973	35.7839***	30.0162***	25.5407**
Western Race		1961	20.0719*	53.1190***	59.7480***
Western Race		1971/72	32.6585***	91.9175***	67.9745***

maintenance of the colour pattern polymorphism of *Chortoicetes*.

4.5a MIGRATION

When a population is not completely isolated from other populations, its gene frequencies may be subject to alteration through the incorporation of migrants, which, as a group, have gene frequencies differing from that of the recipient population. Migration thus tends to reduce differences in gene frequency between the recipient and donor groups.

In particular, migration is very extensive in the eastern race of *Chortoicetes*. Solitary and mass migration are known to occur commonly and the factors governing migration are probably similar in both cases.

Solitary night flight from low density populations (i.e. one to 100 individuals per 1,000 sq. yd. (Clark 1973a)) has been reported in the central west of N.S.W. and channel country of Queensland (Clark, 1969, 1971). It appears that temperature, humidity, wind strength and decreasing light intensity act in concert to exert a threshold effect on flight behaviour (Clark, 1969). Dispersion is non-random, locusts being displaced downwind in convergent wind fields which seems to enhance the likelihood that they are transported into areas of rain. This procedure plays an important role in the population dynamics of the locust since the probability of effective

rain in any one region of the wide distribution area is low. Distinct concentrations of locusts may be formed in areas where numbers were low before migration. The increase in numbers in such areas may be of the magnitude of 37-fold (Clark, 1969a). Moreover, area densities, i.e. average densities over hundreds of square miles, which have resulted from immigration often approach those of young adults which have resulted from local breeding during an outbreak (Clark, unpublished).

Mark-recapture experiments have shown that during the day, individual flights are short and random at low wind speed although sustained day flight from unsuitable areas has been reported (Clark, 1971). Most of the factors governing solitary flight are also responsible for the large scale migration in higher density populations. Slow moving swarms at heights of 50' to 100' above ground during the day were reported by Casimir (1962) whilst Magor (1970) found distinct routes along which extended flight for several hundreds of miles was undertaken by flying swarms, especially during plague periods and usually at night. Lambert (1972) observed that day time flight activity differed qualitatively from that observed at night; it was dependent on environmental conditions rather than the physiological state of the locust. Night flight on the other hand was limited by the insect's physiological state as well as environmental factors.

It is probable that migration in the western race differs from that of the eastern race. Swarming as it is known in the eastern race does not occur and even local breeding, giving rise to 'loose swarms', is extremely rare

and of a local nature (Key, 1938). The solitary counterparts in other locust species have been shown to exhibit marked behavioural differences from their gregarious forms. For example, in *Locustana pardalina* day flight by solitary locusts has been shown to be of short duration and occurs in such a way that the adult locust remains in the same general area (Lea, 1969). However, Botha and Jansen (1969) found that some solitary locusts undergo extended flight at night, resulting in random dispersal. Studies of the western race of *Chortoicetes* have been neglected and the factors governing distribution, behaviour etc are largely unknown.

Mass migration in *Chortoicetes* appears essentially to be induced by over-population brought about, either by a gradual increase of hatchings over deaths during a more or less prolonged period of years, or more usually by a sudden increase in reproductive power which occurs to a prodigious extent at varying intervals of time. From the evolutionary viewpoint then, migration is likely to be an adaptive mechanism operating for the survival of those individuals which remain in the habitat. It is a convenient way of eliminating a high proportion of the numbers in a population when competition for space and food become important for survival. If mass migration resulted in important changes in the population gene pool, it would impose an unnecessary evolutionary load on the population. Several generations of selection would then be required to equilibrate the gene frequencies to levels approaching

those determined by the habitat for the non-migratory population. Samples of migrating and non-migratory locusts have been collected and compared for colour pattern frequency by Byrne (1962) and Davies (unpublished). The evidence of both authors suggested that there is no differential migration with respect to colour pattern. Mass migration in *Chortoicetes* can therefore be regarded as an environmentally determined density regulating mechanism which acts to regulate population density before all resources are depleted and does so quite independently of the genetic structure of the population.

It appears unlikely that migrants found new populations which can be maintained for a long period of time. More reasonably, it would be expected that new populations formed in unfavourable habitats would persist for a small number of generations if climatic conditions permitted. Indeed, an example of this was observed by the author during the course of this study. In the summer of 1970/71 a large scale plague of locusts migrated into and colonised a tract of land in N.S.W. bounded by Hay, Hillston, Darlington Point and Deniliquin. Large numbers of egg laying sites were subsequently detected by officers from pasture protection boards in the district. Significant numbers of locusts hatched in the spring of 1971 and loose swarms of adults were commonly encountered during December of that year. A further migration into the Hay-Deniliquin area occurred in January of 1972 resulting in re-infestation of those areas that had been subjected to control measures

(i.e. insecticide spraying). Egg beds were again detected and this was followed by extensive hatching in spring 1972. Few control measures were undertaken during the 1972/73 season and by the autumn of 1974 numbers of locusts had dwindled to pre-infestation levels. These fluctuations were correlated with favourable weather conditions which occurred from the summer of 1970 to the spring of 1972 although after this period rainfall and other environmental conditions were not so favourably disposed to locust survival and breeding.

Migration in *Chortoicetes* could also be an important factor in changing gene frequencies where the invading immigrant population had different gene frequencies from the local population. In the simple case, if i of the gametes which unite to form the next generation come from immigrants and q_i is the frequency of a gene among the immigrants, whilst q is the frequency of the same gene in the recipient population, then the gene frequency (q) in the next generation of the recipient population is:

$$iq_i + (1-i)q.$$

The change in gene frequency within the recipient group as a result of one generation of such immigration is therefore:

$$q = i(q_i - q).$$

This can be zero only when i = 0, or if $q_i = q$. In the former case there is no immigration, while in the latter the immigrants are qualitatively the same as the natives.

4.5b SELECTION

It has been established that the most general basis for maintaining polymorphism is that in which the heterozygote is at an advantage compared to both homozygotes (Fisher, 1930a).

A common type of calculation which has been applied in the past to population genetics studies has utilised the Hardy-Weinberg Law. In this procedure zygotic frequencies obtained from the relative frequencies of individuals of different genotypes from a sample population are used to calculate gametic frequencies. Expected zygotic frequencies are then calculated by applying the binomial expansion to the observed gametic frequencies and the observed and expected distributions are then compared genotype-by-genotype. Departure from the Hardy-Weinberg expression was subsequently used as evidence for heterosis (Dobzhansky and Levene, 1948; Hexter, 1955; Epling, *et al*, 1957 and Kojima and Yarbrough, 1967). However, Wallace (1958), Novitski and Dempster (1958) and Prout (1965) have shown such analyses to be invalid. For example, it is possible that such deviations are not due to increased viability of the heterozygote(s) but rather to decreased viability of the homozygous class(es).

Fortunately, Fisher's (1930b, 1939) analysis of the grouse locusts is directly applicable to the populations of *Chortoicetes* provided that several assumptions are made. It has thus been possible to re-examine the predictions advanced from Fisher's (l.c.) theory of selective equilibrium in polymorphic species using the collected

random population samples of *Chortoicetes* (Table 4.1). Since it has been established that there is heterogeneity among the colour pattern with respect to sex (4.2a), male and female samples are treated separately.

Two important assumptions are necessary to carry out this analysis. For the first part of the analysis it must be assumed that the colour patterns are determined by a series of linked loci rather than multiple alleles. Secondly, the rubiginosa type is considered as the equivalent of the "universal recessive" of the grouse locusts. There are several logical reasons for doing this. Both the author and Byrne (1962, 1967a) consider the rubiginosa gene to be the most recessive (least dominant) of the genes at the F locus, the contrasting patterns being incompletely dominant over the cryptic uniformly mottled rubiginosa pattern. Rubiginosa differs from the universal recessive in that only the albomedia gene retains complete dominance over it such that A/A and A/R are phenotypically indistinguishable. The R/T and N/R forms show a combination of features of the respective homozygous patterns. The rubiginosa pattern also parallels (though not at the same magnitude) the universal recessive in that it is the most frequently occurring gene in all populations that have been studied by the author (e.g. Table 4.9) and other workers (Key, 1954; Byrne, 1967b; Davies, unpublished). Furthermore, an examination of the insects in the C.S.I.R.O. Museum revealed that the rubiginosa pattern is the only one that

is common to *Chortoicetes* and the nine closely related *Austroicetes* species. It also appears probable that this pattern is the most abundant form in the *Austroicetes* spp. (Key, personal communication).

The western race samples have been included in the analysis although the findings are somewhat limited for it because of the heterogeneous nature of the population samples (i.e. adults and nymphs). All other samples are adults. As stated in Chapter 3, the genotypes A/A and A/R are not distinguishable phenotypically in the field. However, the albomedia type is not very frequent in most populations, and with random mating the proportion of homozygotes is likely to be negligible in comparison with the heterozygotes. For example, if R=0.5 and A=0.01 the proportion of A/R:A/A would be 100:1. In the following calculations all individuals classified under A/A:A/R in table 4.1 were assumed to have the A/R genotype.

Although it is possible to follow the ensuing analysis, the reader is referred to Fisher (1939, p.110 *et seq*) for the precise statistical theory concerned. Taking as an example the Hay 1971 sample, the frequencies for the different dominant genes are shown in column 2 of table 4.11 (see Appendix 4.2 for the other populations). Column 3 of the table represents the cumulative frequency (N+T+A) while column 4 is obtained by multiplying the frequencies of N,T,A one with the other, summing the resultant products. The final column represents the frequencies multiplied successively (NxTxA). The values thus obtained are now

TABLE 4.11

Elementary Symmetric Functions for Hay 1971

	Dominant	Frequency	Cumulative	Cumulative Product	Successive Product
Male	N	554	554		
	T	417	971	23,018	
	A	3	974	233,931	693,054
				162.5649	0.3347
Female					
	N	370	370		
	T	302	672	111,740	
	A	7	679	116,444	782,180
				111.1107	0.7122

divided by n (column 4) and n^2 (column 5) giving the distribution shown in table 4.12 and appendix 4.3.

It is now possible to compare the observed frequencies with those expected in the absence of selection against compounds of two or more dominants (Table 4.13 and Appendix 4.4). In essence, 0 refers to the number of observed and expected rubiginosa (R/R) individuals; 1 is the number of R/N + R/T + R/A; and 2+3 equals A/N + A/T + N/T + N/N + T/T. As with Fisher, the compounds of 2 and 3 are placed in the one class, the expectation of compounds of three dominant genes being necessarily low (Table 4.12, Appendix 4.3) and none were recorded in the collected samples. However, such individuals have been observed in the wild and the laboratory. The laboratory specimens showed features attributable to ANT (three specimens) whilst the wild specimen was classified as RNT (collected by R.A.H. Davies). If the classification of these individuals is correct then the multiple allelic hypothesis cannot be true. Attempts to breed from these individuals were unsuccessful as they died before mating.

The chi-square values of 135 and 68 (Table 4.13) are clearly significant. Only one degree of freedom is used since the total number of observations as well as the total number of dominants is used in calculating the expectation. Table 4.14 shows the chi-square and probability levels for each population - full details are provided in appendix 4.4. Out of the 22 comparisons available 16 show a distinct excess of the one dominant class

TABLE 4.12

Frequency Distribution Expected

for the genotypes of the Hay 1971 population

	0 (R/R)	1 (R/N+R/T+R/A)	2 (A/N+A/T+N/T +N/N+T/T)	3 (Compounds of three dominants)
Male				
	1439			
	-974	974		
	162.5649	-325.1298	162.5649	
	- 0.3347	1.0041	- 1.0041	0.3347
	627.2302	649.8743	161.5608	0.3347
Female				
	1048			
	-679	679		
	111.1107	-222.2214	111.1107	
	- 0.7122	2.1366	- 2.1366	0.7122
	479.3985	458.9152	108.9741	0.7122

TABLE 4.13

Observed versus Expected Comparison

of the number of dominants for the Hay 1971 population

Male	Expected (m)	Observed (m+x)	Difference (x)	X^2/m
0	627.23	454	-173.23	47.8431
1	649.87	870	+220.13	74.5645
2+3	161.89	115	-46.89	13.5813
	1438.99	1439		135.9889
Female				
0	479.39	361	-118.39	29.2376
1	458.92	591	+132.08	38.0134
2+3	109.68	96	-13.68	1.7063
	1047.99	1048		68.9573

TABLE 4.14

Chi-Square and Probability Values for all Populations testing the observed and expected number of dominants

POPULATION	SEX	CHI-SQUARE	PROBABILITY LEVEL
Hay	Male	352.68	****
(Autumn) 1972	Female	369.23	****
Boulia	Male	996.53	****
(Autumn) 1973	Female	690.32	****
Coonamble	Male	45.03	****
(Spring) 1960	Female	43.72	****
Trangie	Male	58.16	****
(Autumn) 1973	Female	33.36	****
Coona	Male	7.17	**
(Autumn) 1973	Female	657.16	****
White Cliffs	Male	1.05	$0.3 < p < 0.5$
(Autumn) 1973	Female	19.52	****
White Cliffs	Male	35.90	****
(Spring) 1973	Female	3.01	$p > 0.05$
Tibooburra	Male	4.05	*
(Spring) 1973	Female	4.04	*
Western Race	Male	53.26	****
1961	Female	48.62	****
Western Race	Male	8.95	**
1971/72	Female	92.34	****

(i.e. R/A, R/N, R/T) (Table 4.13, Appendix 4.4). Apparently then, there is a selective aversion of the dominants with each other and in the homozygous class (i.e. N/T, N/A, A/T and N/N, T/T) in most cases.

Fisher (1939) has suggested that 'owing to the very close linkage found in *Paratettix texanus* quite small selective intensities would suffice to maintain the frequency of coupled pairs at so low a level that the frequencies in nature should be indistinguishable from those of a population showing variation in only a single series of multiple allelomorphs. The theoretical relationship which holds among the frequencies in such a sytem may be deduced by equating the frequency of the recessive gamete to the square root of the recessive zygote.'

For example, considering the male sample from Hay, there are 454 R/R out of a total of 1381 (ignoring N/N, T/T for the current purpose of analysis). Therefore, if p is the frequency of gametes containing rubiginosa as the only factor

$$\sqrt{454} = p\sqrt{1381}.$$

Taking nigrovirgata, there are 497 insects of the genotype N/R; hence, if q is the frequency of gametes bearing N only, p+q is the frequency of gametes recessive for all factors but N and the corresponding equation is

$$\sqrt{951} = p + q\sqrt{1381}$$

A similar equation may be derived for the trilineata and albomedia genes. In a multiple allelomorphic system, the sum of p, q..... must add up to one. To test this, the quantities corresponding to the left hand side of the derived equations are added and the figure $2\sqrt{454} + \sqrt{1381}$ subtracted (Appendix 4.5).

This analysis revealed that none of the solved equations summed to unity providing further circumstantial evidence for the existence of a linked series of loci as opposed to multiple alleles (Table 4.15.) Eighteen of the 22 calculations resulted in a positive difference, indicating that the dominant compounds observed in the wild are generally less frequent than they should be, even in the complete absence of combinations in coupling. As an example, the deficiency for the Hay sample may be calculated by squaring the difference of 80.9001 - 42.6144 giving 1466.4840 which exceeds 1381 (the total) by 85.4840. Therefore, about 85 more compounds would be needed to agree with the theory. A comparison of the observed and expected number of compounds for all populations is presented in table 4.16.

Fisher (1939) attributed the absence of coupling type gametes to the selective elimination of the dominant compounds. The deficiencies observed in table 4.16 indicate that selection in the past has left its mark on the species by eliminating chromosomes containing more than one dominant. During the actual generation sampled selection has eliminated a large proportion of the double dominant heterozygotes

TABLE 4.15

Test for multiple allelomorphism among various natural populations

Hay 1971 (Spring)		Hay 1972 (Autumn)		White Cliffs 1973 (Autumn)	
Male	Female	Male	Female	Male	Female
+1.1329	+0.6960	+9.3396	+9.9760	+0.450	-0.03396

White Cliffs (1973) Spring		Tiboobürra (1973) Spring		Trangie 1973 (Autumn)	
Male	Female	Male	Female	Male	Female
-0.3461	+2.9501	+4.5406	+3.6832	+0.7518	+0.4666

Boulia 1971 (Autumn)		Coonamble 1960 (Spring)		Coona 1973 (Autumn)	
Male	Female	Male	Female	Male	Female
+3.5182	+2.3834	-0.4373	-0.6157	+0.1821	+0.2157

Western Race 1961		Western Race 1971-72	
Male	Female	Male	Female
+0.2863	+0.2848	+0.4483	+0.7999

TABLE 4.16

Comparison of Number of Dominant Compounds Observed and Expected

POPULATION	SEX	TOTAL INSECTS	OBSERVED	EXPECTED	DIFFERENCE	% OBS/ EXP.
Hay 1971	Male	1439	57	142.4840	-85.4840	40.0004
(Spring	Female	1048	44	88.4108	-44.4108	49.7677
Hay 1972	Male	2728	80	31.9909	+48.0091	250.0711
(Autumn)	Female	2781	81	34.8984	+46.1016	232.1023
Boulia 1972	Male	1087	21	226.8433	-205.8433	9.2575
(Autumn)	Female	1428	27	191.6329	-164.6329	14.0894
Coonamble	Male	592	83	63.4930	+19.4069	130.5175
1960	Female	553	67	38.8408	+28.1591	172.4990
(Spring)						
Trangie	Male	1214	6	58.1714	-52.1714	10.3143
1973	Female	510	17	37.5521	-20.5521	45.2704
(Autumn)						
Coona 1973	Male	472	5	12.8163	-7.8163	39.0128
(Autumn)	Female	307	4	11.5417	-7.5417	34.6569
White Cliffs	Male	402	0	1.8020	-1.8020	
1973	Female	749	35	16.8494	+18.1506	207.7225
(Autumn)						
White Cliffs	Male	234	16	5.9708	+10.0292	267.9708
1973	Female	112	13	4.0044	+8.9956	324.6429
(Spring)						
Tibooburra	Male	181	4	7.3052	-3.3052	54.7555
1973	Female	109	13	5.3748	+7.6252	241.8695
(Spring)						
Western Race	Male	185	19	26.1180	-7.1180	72.7468
1961	Female	138	11	17.1090	-6.1090	64.2936
Western Race	Male	74	6	13.4286	-7.4286	44.6808
1971/72	Female	78	2	14.1761	-12.1761	14.1083

in repulsion which must be formed afresh by chance in each generation. The consistent deficiency of insects carrying more than one dominant, compared with expectation (based on the supposition that combinations in coupling are completely absent) is thus of considerable importance. Considering the results for all samples (Table 4.16) the number of compounds observed, 611, is approximately 58 per cent of the expectation, i.e. some 42 per cent of dominant compounds are eliminated on average in a single generation between fertilisation and adulthood. Comparing the two races, there is little difference, the figures being 41.34 and 46.36 per cent elimination for the eastern and western races. These figures compare favourably with Fisher's estimate of 40 per cent elimination in *Paratettix texanus*.

An explanation of the above phenomenon is possible if it is assumed that a 'single' heterozygote possesses an advantage over the respective homozygous forms and that, individuals heterozygous for two loci also suffer some disadvantage. For example, the genotype N/R ($\frac{N}{+}$) would be at an advantage over N/N ($\frac{N}{N}$) and R/R ($\frac{+}{+}$) (similarly for A/R, R/T, A/N, A/T, N/T and their respective homozygotes), whilst N/R ($\frac{N}{+}$), A/R ($\frac{A}{+}$) and R/T ($\frac{+}{T}$) (i.e. "single" dominants) would be preferred over A/N, A/T or N/T (i.e. double dominants).

If the colour patterns were in fact determined by linked loci then under these circumstances very strong selection in favour of any mechanism suppressing crossing over between any two of the loci would be expected.

Consider an individual with the chromosome constitution N/T, a cross over between the N and T loci would produce the chromosomes NT and ++ on a two locus model or aNT and ant on a three locus model. The double dominant coupling phase chromosome (NT) would be severely handicapped because of the intense selection pressure directed against it. Calculations performed in this chapter indicate a selection pressure in the vicinity of 40 per cent on average. Alternatively, a cross over in a double heterozygote of the constitution $\frac{NT}{++}$ would be advantageous, since it would produce the chromosomes N+ and +T. Double heterozygotes of this type must be extremely rare because of their distinct disadvantage in the population so it is probable that the coupling phase is practically non-existent. Thus the genes, advantageous by themselves, interact with one another in a manner such that in combination they are disadvantageous.

4.6 FECUNDITY/VIABILITY OF THE COLOUR PATTERNS

If the genic series constitutes a selectively balanced polymorphism, then the conditions required for a stable equilibrium demand that no heterozygote be less fit than both the associated homozygotes (Wright, 1969). In order to compare the viability of homozygous and heterozygous forms matings between the different pattern types were set up.

General laboratory maintenance procedures have been outlined in 2.4. To enable the collection of a large amount of information (thus increasing the accuracy

of the fecundity and viability estimates) mass matings of each colour pattern type were established. Each egg pod was placed in a separate container for incubation and the hatchlings were examined before second instar, when colour patterns and sex were recorded.

The mean number of hatchlings per pod (categorised by sex) was calculated for each colour pattern mating type (Table 4.17, Appendices 3.1 - 3.17). A one-way analysis of variance (ANOVA) was carried out for each of the colour pattern groups (Table 4.18). It is immediately apparent that there is variability within the homozygous and heterozygous classes of the eastern race. However, no difference exists within the same categories of the western race. Significant differences between particular colour pattern types may be referred to in table 4.19. In both sexes of the eastern race the number of hatchlings of the R/R genotype was significantly higher than the other homozygous colour pattern types (i.e. N/N, T/T, A/A) which did not differ amongst themselves. The heterozygotes can be separated into two classes (A/N, A/T, N/T and A/R N/R, R/T) which are generally homogeneous within themselves but differ significantly between the two classes.

Examination of the numbers for each sex, by colour pattern, revealed that there was very little difference between the means (Table 4.20). The numbers were thus amalgamated and weighted means were calculated for each of four classes (Table 4.21). There was no significant

TABLE 4.17

Mean Number of Hatchlings Per Pod

EASTERN RACE							WESTERN RACE						
Colour Pattern	Appen-dix	Number of Egg Pods	Male		Female		Colour Pattern	Appen-dix	Number of Egg Pods	Male		Female	
			Mean	S.D	Mean	S.D				Mean	S.D	Mean	S.D
R/R	3.3	50	12.620	5.649	11.620	4.690	R/R	3.11	15	9.733	4.183	9.267	5.230
A/A	3.1	39	8.487	3.852	7.769	4.023							
N/N	3.2	32	8.594	4.543	9.250	4.080	N/N	3.12	10	11.200	3.190	10.400	4.061
T/T	3.4	46	8.391	4.602	7.913	4.713	T/T	3.13	8	8.625	6.209	6.000	3.024
A/R	3.6	40	9.426	8.522	9.876	7.726	A/R	3.15	13	6.692	6.272	8.230	7.118
N/R	3.8	28	8.322	7.206	9.501	8.010	N/R	3.16	3	11.667	7.107	14.667	7.713
R/T	3.10	38	10.473	7.710	10.605	7.317	R/T	3.17	43	10.836	8.481	10.512	10.512
A/N	3.5	20	10.800	6.747	11.199	7.965	A/N	3.14	9	8.112	6.903	8.445	5.766
A/T	3.7	7	8.001	9.504	9.000	10.776							
N/T	3.9	43	12.930	9.984	11.976	8.142							

TABLE 4.18

Analysis of Variance of Homozygote and Heterozygote Groups

Source of Variation	df	Sum of Squares	Mean Square	Variance Ratio	
EASTERN RACE					
Homozygotes					
male					
treatment	4	839.88282	209.97071	9.73	**
error	180	3885.97664	21.58876		
female					
treatment	4	581.17238	145.29309	7.56	**
error	180	3458.63303	19.21463		
Heterozygotes					
male					
treatment	5	228.18155	45.63631	4.81	**
error	170	4576.24467	9.49428		
female					
treatment	5	146.25405	29.25081	3.47	**
error	170	4059.63940	8.42249		
WESTERN RACE					
Homozygotes					
male					
treatment	2	30.31894	15.15947	0.75	
error	30	606.40833	20.21361		
female					
treatment	2	91.57576	45.78788	2.31	
error	30	595.33333	19.84444		
Heterozygotes					
male					
treatment	3	20.43221	6.81074	0.88	
error	52	1451.02329	7.75948		
female					
treatment	3	39.30790	13.10263	1.58	
error	52	1553.86481	8.30944		

TABLE 4.19

Table of Differences between Genotypes Within Homozygous and Heterozygous Groups of the Eastern Race

Male

Homozygotes	N/N	R/R	T/T
A/A		***	
N/N		***	
R/R			**

Heterozygotes	A/T	N/T	A/R	N/R	R/T
A/N			*		
A/T		*			
N/T	*			***	*
A/R	**			***	**
N/R		***	***		

Female

Homozygotes	N/N	R/R	T/T
A/A		**	
N/N		*	
R/R			***

Heterozygotes	A/T	N/T	A/R	N/R	R/T
A/N			*		
A/T		*	*	***	**
N/T	*		*	*	
A/R	*	*		***	**
N/R	***	*	***		

TABLE 4.20

Values of t for Test Between Means of Sexes with Respect to Genotype

	Eastern Race		Western Race	
	t		t	
R/R	2.1770	*	0.5682	
A/A	1.5957		-	
N/N	1.2285		0.8912	
T/T	1.0502		2.2856	*
A/R	0.6967		1.4557	
N/R	1.5703		1.1019	
R/T	0.2070		0.4817	
A/N	0.4531		0.2643	
A/T	0.5432			
N/T	1.4517			

TABLE 4.21

Mean Number of Hatchlings per Pod Scored at Late First Instar

Colour Pattern	Eastern Race			Western Race		
	Mean	S.D.	n	Mean	S.D.	n
A/A	16.256	7.875	39			
N/N	17.844	8.623	32	21.600	7.251	10
R/R	24.240	10.339	50	19.000	9.413	15
T/T	16.304	9.315	46	14.625	9.233	8
A/N	21.999	14.712	20	16.557	12.669	9
A/T	17.001	20.280	7			
N/T	24.906	18.126	43			
A/R	19.102	16.248	40	14.922	13.390	13
N/R	17.823	15.216	28	26.334	14.820	3
R/T	21.078	15.027	38	21.348	17.229	43

Weighted Means by Groups

R/R	24.240	10.339	50	19.000	9.413	15
A/A, N/N, T/T	16.709	8.646	117	18.500	8.131	18
A/N, A/T, N/T	23.284	17.365	70	16.557	12.669	9
A/R, N/R, R/T	19.472	15.537	106	20.185	16.260	59

difference between the groups of the western race (Table 4.22). In the eastern race, however, the homozygous group A/A, N/N, T/T had a significantly lower mean number of hatchlings than did the R/R and A/N, A/T, N/T groups (Table 4.22). Between race comparisons by colour pattern revealed no differences (Table 4.22).

Matings of the generalised type P/Q x P/Q (i.e. A/N x A/N, A/T x A/T, N/R x N/R, R/T x R/T, N/T x N/T) in *Chortoicetes* enable unequivocal classification of the homozygous and heterozygous classes (with the exception of A/R x A/R). Segregation of colour patterns for each egg pod scored may be referred to in appendices 3.5 to 3.10 and 3.14 to 3.17.

Examination of the observed and expected numbers for the cumulative totals of matings of type P/Q x P/Q in the Appendices revealed that the number of heterozygotes observed was generally higher than expected. Provided there are no viability differences, the number of homozygotes should equal the number of heterozygotes in matings of the type P/Q x P/Q. On this basis, comparison of the observed frequencies for each of the relevant pattern type matings should indicate if there are any differences in viability at the time of classification. Table 4.23 gives the observed cumulative frequencies for each mating type.

In total, 1332 homozygotes and 1598 heterozygotes were identified for the eastern race, the percentage viability of the homozygotes being 82.7 per cent of the

TABLE 4.22

Within Race Comparison by Colour Pattern

(t-test)

COMPARISON	Eastern Race			Western Race		
	t	d.f.	P	t	d.f.	p
R/R : A/A, N/N, T/T	4.817	167	**	0.157	31	
R/R : A/N, A/T, N/T	0.340	118		0.514	22	
R/R : A/R, N/R, R/T	1.958	154		0.266	72	
A/A, N/N, T/T: A/N, A/T, N/T	3.445	185	**	0.462	25	
A/A, N/N, T/T : A/R, N/R, R/T	1.595	221		0.415	75	
A/N, A/T, N/T : A/R, N/R, R/T	1.473	174		0.628	66	

Between Race Comparison by Colour Pattern

R/R: R/R	1.728	63	
A/A, N/N, T/T : N/N, T/T	0.817	133	
A/N, N/T, A/T : A/N	1.109	77	
A/R, N/R, R/T : A/R, N/R, R/T	0.270	164	

TABLE 4. 23

Cumulative Numbers for Each Mating Type

EASTERN RACE

Genotype	A/A	A/N	A/T	N/N	N/R	N/T	R/R	R/T	T/T	No. of Matings	No. of Progeny
A/N x A/N	97	225		118						20	440
A/T x A/T	29		62						28	7	119
N/R x N/R				99	300		100			28	499
N/T x N/T				252		574			245	43	1071
R/T x R/T							192	437	172	38	801
Observed	126	225	62	469	300	574	292	437	445	136	2930
Expected	139.75	220	59.5	502.5	249.5	535.5	325	400.5	497.5		
% Excess / Deficiency	-10%	+2%	+4%	-7%	+20%	+7%	-10%	+9%	-11%		

WESTERN RACE

Genotype	A/A	A/N		N/N	N/R		R/R	R/T	T/T		
A/N x A/N	35	74		41						9	150
N/R x N/R				20	41		18			3	79
R/T x R/T							190	470	258	43	918
Observed	35	74		61	41		208	470	258	55	1147
Expected	37.5	75		57.25	39.5		249.25	459	229.5		
% Excess / Deficiency	-7%	-1%		+6%	+4%		-17%	+2%	+12%		

heterozygous class. The western race difference in viability deficiency (562 homozygotes vs 585 heterozygotes - 96.0 per cent) is less marked. Individual colour pattern viabilities may be referred to in table 4.24. The inferior viability of the homozygous classes indicates that the polymorphism may be maintained in part by heterozygote advantage.

Re-casting the data as in table 4.25 enables a more complete analysis. In both races the observed values are significantly different from expected. Comparing the observed and expected frequencies (in the eastern race) there appears to be a selective advantage expressed in terms of the mean number of hatchlings of N/R, R/T:N/T A/T, A/N:N/N, A/A, T/T:R/R. In the western race the grouping is slightly different being T/T, N/N, A/A:N/R, R/T:A/N:R/R. This may indicate that the selective forces maintaining the polymorphism in the western race are different to those of the eastern race, or more likely it reflects a bias in the data since the number of egg pods and nymphs used for the western race estimate is small (with the exception of the R/R, R/T, T/T genotypes). If the 'single' dominant heterozygote (N/R, R/T) class is allocated a value of 1 then the relative excess/deficiencies from table 4.25 are:-

	Eastern Race	Western Race
Double Dominant Heterozygotes (i.e. N/T, A/T, A/N)	0.94	0.96
Dominant Homozygotes (i.e. N/N, T/T, A/A)	0.80	1.06
'Universal Recessive' (i.e. R/R)	0.79	0.80

TABLE 4.24

Individual Genotype Viabilities

(N/R = 1.0)

Genotype	Eastern Race			Western Race		
	Relative Viability	Estimate Based On		Relative Viability	Estimate Based On	
		No. of Egg Pods	No. of Nymphs		No. of Egg Pods	No. of Nymphs
A/A	0.75	27	559	0.89	9	150
N/N	0.78	91	2010	1.02	12	229
T/T	0.74	88	1991	1.08	43	918
R/R	0.75	66	1300	0.79	46	1467
R/T	0.91	38	801	0.98	43	918
N/R	1.00	28	499	1.00	3	79
A/N	0.85	20	440	0.95	9	150
A/T	0.87	7	119			
N/T	0.89	43	1071			

TABLE 4. 25

Summarised Data for Matings of the

Generalised Type P/Q x P/Q

Eastern Race

	R/R	N/R, R/T	A/A, N/N, T/T	A/T, N/T, A/N	n
	100	300	99		499
	192	437	172		801
			497	574	1071
			57	62	119
			215	225	440
Observed	292	737	1040	861	2930
Expected	325	650	1139.75	815	
% Excess / Deficiency	-10%	+13%	-9%	+6%	

$X^2_3 = 26.3216$ **

Western Race

	18	41	20		79
	190	470	258		918
			76	74	150
Observed	208	511	354	74	1147
Expected	249.25	498.5	324.25	75	
% Excess / Deficiency	-17%	+3%	+9%	-1%	

$X^2_3 = 9.8706$ *

Although selective intensities of the order above certainly exist in the wild (Fisher, 1930b, 1931; 4.5b) it is not known to what causes they are ascribable to in *Chortoicetes*. A difference in the fecundity of the colour patterns has already been demonstrated and this coupled with accurate measurements of relative viabilities (q.v. 4.7) should provide some insight into the maintenance of the polymorphism.

4.7 EXPERIMENTAL DETERMINATION OF COLOUR PATTERN VIABILITIES

The estimation of fecundity and viability for different colour patterns based on breeding data has already been considered (4.6). In order to assess further the physiological bases responsible for maintenance of the colour pattern polymorphism, a study on the effect of different environments on genotypes was initiated. With precise data on relative viabilities, fecundities and fertilities, it may be possible in the future to infer the magnitude of selective advantage which in nature favours different genotypes.

In this study, temperature, photoperiod and food quality were varied systematically. Since rainfall is particularly correlated with locust numbers (Casimir, 1962; Clark *et al*, 1969; Clark, 1973a, b), a difference in survival rate would be expected '*a priori*' between nymphs reared on dry food and those reared under optimal conditions. It is important to know whether the different survival rate is reflected differentially amongst the genotypes.

METHODS

Wheat was the stable food source. Nymphs were scored for colour pattern and sex from known matings of laboratory stocks late in first instar. Known numbers were then set up at equivalent densities (150 per cage, representing field swarming densities) in cage type A for each of five experimental designs.

Experimental Designs

	Treatment				
	I	II	III	IV	V
Temperature °C	33°/27°	33°/27°	33°/27°	33°/27°	27°/21°
Photophase hrs	15½	11	15½	11	15½
Food Source	wet	wet	dry	dry	wet

The cabinets used were described in 2.4. Dry wheat was obtained (following Davies, unpublished) by transferring wet wheat (about 3" high) to a constant temperature room of 33°C ± 1°C which was illuminated constantly. Three days later, the wheat was transferred to the dry cage condition. This process was repeated at four day intervals for the dry food source, whereas wet food was supplied *ad libitum*. Individual locusts were removed and scored after they fledged.

RESULTS

(i) WATER CONTENT OF THE FOOD

I am indebted to Mr. R.A.H. Davies for allowing the use of information quoted in this paragraph. He calculated the mean percentage water content of green wheat as 98.4 per cent (10 measurements); and that of dried wheat

six days after introduction 19.1 per cent (n=10). Although the dried and wet wheat had the same initial water content, the dried wheat was chlorotic in appearance and it had passed the permanent wilting point. Locusts reared in wet wheat cages thus had a constant supply of moisture in their diet whilst the dry cage locusts had a fluctuating supply of moisture.

(ii) EXPERIMENTAL DETERMINATION OF GENOTYPE VIABILITIES

The actual numbers of each genotype set up and fledged for each sex may be referred to in table 4.26. The usual arcsin transformation of the data was used as the normalising factor resulting in the removal of absolute differences in numbers. In the initial analysis, no pattern in the Experimental Design could be used and so the analysis was a two-factor analysis with several empty cells (i.e. 15 missing values). The analysis of variance revealed significant effects of both treatment and genotypes for each sex at the gross level (Table 4.27).

Partitioning the information firstly at the treatment level (Appendix 4.6) the differences outlined in table 4.28 are found. Out of four comparisons where the experimental variable was photoperiod, three showed no significant difference from each other. The only difference was the female dry I versus dry II where the large number of missing values probably resulted in a biased estimate. No overall difference was detected between western race locusts reared under a photophase of 11 hours and the

TABLE 4.26

Numbers of Each Genotype Set up and Fledged under Different Environmental Conditions

GENO-TYPE	Room I Optimal		Room 2 Optimal		Room 1 Dry		Room 2 Dry		Room I Optimal 27°/21°		Western Race Room 2 Optimal	
	Set Up	Fledged	Set Up	Fledged	Set Up	Fledged	Set Up	Fledged	Set Up	Fledged	Set Up	Fledged
MALE												
A/A	154	28	57	10	117	19			38	1	10	2
N/N	139	29	71	19	24	1	141	11	110	5	112	20
R/R	119	48	145	52	135	15	92	3	183	62	184	30
T/T	114	32	155	38	60	1	151	2	165	6	148	6
N/R	45	16	91	31	12	2	18	2	34	21	55	15
R/T	121	44	99	41	117	15	40	3	60	21	299	47
A/R	116	26	32	5	94	10	183	7	91	17	81	21
A/N	71	6	24	3	65	5			23	2	49	12
A/T	41	5	18	3	11	2						
N/T	61	9	64	12	14	1	140	2	62	18	17	1
R/P	90	16	123	24	-	-	39	8	49	3		
N/P	95	9	73	11	56	3			35	4		
P/P	96	17	55	7	-	-			18	0		
A/P	166	21	73	7	41	5						
	1428	306	1070	263	746	79	804	38	868	150	955	154
FEMALE												
A/A	123	28	60	11	105	14			27	1	4	1
N/N	114	33	105	26	20	1	127	11	102	3	102	19
R/R	122	51	157	62	130	16	86	2	179	46	156	31
T/T	121	32	166	39	63	2	163	1	135	7	149	4
N/R	55	23	90	27	17	2	22	1	48	21	54	15
R/T	106	29	112	36	113	11	34	2	63	28	278	69
A/R	102	22	29	6	77	12	194	7	84	15	100	26
A/N	73	10	25	3	68	7			24	2	44	15
A/T	13	2	25	5	9	1			-	-	-	-
N/T	62	10	54	11	21	1	104	2	49	13	18	2
R/P	93	25	112	31			37	4	60	4		
N/P	107	14	73	9	63	4			58	4		
P/P	74	11	45	4					27	1		
A/P	162	21	72	4	42	7			-	-		
	1327	311	1125	274	728	78	767	30	856	145	905	182

TABLE 4.27

ANOVA Considering the Effect of Genotype and Environmental Condition

MALE

Source of Variation	df	SS	MS	VR
Treatments ignoring genotypes	5	1353.21354		
Treatments eliminating genotypes	5	1750.33185	350.06637	6.80***
Genotypes ignoring treatments	13	1664.86263		
Genotypes eliminating treatments	13	2061.98094	158.61392	3.08**
Interaction eliminating treatments and genotypes	50	2574.08387	51.48168	

FEMALE

Treatments ignoring genotypes	5	2018.76088		
Treatments eliminating genotypes	5	2373.95989	474.79198	11.56***
Genotypes ignoring treatments	13	1175.76109		
Genotypes eliminating treatments	13	1530.96010	117.76616	2.87**
Interaction eliminating treatments and genotypes	50	2053.83256	41.07665	

TABLE 4.28

Differences Between Treatments Considering all Genotypes

MALE

Experimental Condition			II	III	IV	V	Western Race II	Mean
		Photophase (hours)	11	15½	11	15½	11	
	Wheat		Wet	Dry	Dry	Wet	Wet	
I	Wet	15½		**	***			26.7357
II	Wet	11		***	***			27.1345
III	Dry	15½						17.3850
IV	Dry	11				**	**	11.3095
V 27°/21°	Wet	15½						21.7736
Western Race II	Wet	11						22.0330

FEMALE

I	Wet	15½		***	***	**		28.3207
II	Wet	11		***	***	*		26.7761
III	Dry	15½			**		*	17.5455
IV	Dry	11				***	***	9.8148
V 27°/21°	Wet	15½						21.3418
Western Race II	Wet	11						24.6748

eastern race locusts reared at 11 and 15½ hours photophase on wet food. In summary,photoperiod appears to have little effect on the overall survival of locusts reared under laboratory conditions. In all cases there were differences between the wet and dry treatments. A decrease in temperature also resulted in a lowered survival rate compared with locusts reared at the higher temperature. This was only detectable in females.

In the second part of the analysis the optimal and dry conditions were considered by amalgamating the two different photoperiods for each treatment. ANOVA of the data is presented in table 4.29. Since, for both the male and female, the condition by genotype interaction is significant, a table of genotype means for each level of condition (with appropriate L.S.D's) is presented for each sex (Table 4.30). There are eight missing values estimated in this analysis and the effect of this is to increase the significance of the factors. This was allowed for by using the formula developed in section 7.18 of Cochran and Cox (1964).

Considering table 4.30, there are two distinct groups of genotypes which do not vary with the condition. Of the eight genotypes containing albomedia, only one (A/R male) showed a significant difference between the two conditions. Porphyrica genotypes (with the exception of the homozygote) are also able to survive reasonably well under dry conditions. Thus porphyrica and albomedia genotypes appear to be more viable under dry conditions

TABLE 4.29

ANOVA Between Dry and Wet (Optimal) Conditions

MALE

Source of Variation	df	SS	MS	Reference Error MS	df	VR	
Room	1	68.29957	68.29957				
Condition	1	1657.03893	1657.03893	73.87053	1	22.43	*
Genotype	13	1170.60645	90.04665	9.30681	18	9.68	***
Condition x Genotype	13	951.03589	73.15661	9.30681	18	7.86	***

FEMALE

Source of Variation							
Room	1	200.51247	200.51247				
Condition	1	2211.26900	2211.26900	70.23668	1	31.48	*
Genotype	13	892.51306	68.65485	9.53583	18	7.20	***
Condition x Genotype	13	734.54795	561.50369	9.53583	18	5.93	***

TABLE 4. 30

Genotype Means for Each Level of Condition

L.S.D. 10.275 = 5% *
14.092 = 1% **

Condition	Male Genotype Means													
	A/A	N/N	R/R	T/T	N/R	R/T	A/R	A/N	A/T	N/T	R/P	N/P	P/P	A/P
Optimal	25.001	29.165	38.108	30.836	36.156	38.571	25.771	18.802	22.267	24.124	25.576	20.384	22.894	21.610
Dry	21.512	13.999	14.937	7.013	21.783	18.438	15.157	13.849	22.987	11.183	29.184	11.130	7.597	18.186
Optimal versus Dry		**	**	**	**	**	*			*			**	

L.S.D. 10.317 = 5% *
14.149 = 1% **

	Female													
Optimal	26.925	31.196	39.608	29.971	36.751	33.037	27.364	20.995	24.829	25.254	31.486	20.881	20.012	17.368
Dry	18.405	15.018	14.655	7.378	16.185	16.108	17.101	17.502	16.459	10.288	22.208	11.582	7.558	21.083
Optimal versus Dry		**	**	**	**	**				**			*	

relative to the N/N, R/R, T/T, N/R, R/T and N/T genotypes which are favoured by wetter conditions. The latter genotypes are all significantly less viable under dry conditions as compared with wet conditions.

Further comparison of the genotypes within conditions is made possible by a further analysis (Tables 4.31, 4.32). For this, no pattern in the condition factor could be utilised so a two factor analysis with several empty cells (i.e. 15 missing values) was again carried out. For ease of interpretation a non-orthogonal analysis was used resulting in no bias in the variance ratio since no missing values were estimated. There is also no error term for the testing of the interaction and thus the interaction becomes the "error" for testing main effects.

Considering the wet conditions there appears to be a separation into groups which are characterised by similar viabilities. Thus, R/R, R/T, N/R have a higher viability compared with N/N, T/T which enjoy an advantage over A/N, A/T, N/T. The latter comparison is not as clear in the female sex. Porphyrica genotypes generally have the lowest viability under this experimental condition. Moreover, the grouping effect is not immediately noticeable under dry conditions. The feature of table 4.32 is the marked increase in the viability of the R/P genotype and decrease in viability of the T/T genotype. Thus it appears that there is differential viability of genotypes under the same and different environmental conditions.

TABLE 4. 31

Differences Between Genotype Viabilities when Locusts Reared under the Same Environmental Condition. 1. Wet

MALE

Genotype	R/R	N/N	T/T	N/R	R/T	A/R	A/N	A/T	N/T	R/P	N/P	P/P	A/P
A/A	**			**	**								
N/N	**			*	**		**	*	**				*
T/T	*				*		**	*	*		**	*	**
R/R						**	**	**	**	**	**	**	**
N/R						**	**	**	**	**	**	**	**
R/T						**	**	**	**	**	**	**	**
A/R							*						
A/N										*			
A/T													
N/T													
R/P													
N/P													
P/P													

FEMALE

Genotype	R/R	N/N	T/T	N/R	R/T	A/R	A/N	A/T	N/T	R/P	N/P	P/P	A/P
A/A	**			**									**
N/N	*			*			**				**	**	**
T/T	**						**				**	**	**
R/R						**	**	**	**	*	**	**	**
N/R						**	**	**	**		**	**	**
R/T							**	*	*		**	**	**
A/R												*	**
A/N										**			
A/T										*			*
N/T													*
R/P											**	**	**
N/P													
P/P													

TABLE 4. 32

Differences Between Genotype Viabilities when Locusts Reared Under the Same Environmental Condition. 2. Dry

MALE

Genotype	N/N	T/T	R/R	N/R	R/T	A/R	A/N	A/T	N/T	R/P	N/P	P/P	A/P
A/A	*	**	*				*		**	*	**	**	
N/N		*		*				**		**			
T/T			*	**	**	*	*	**		**			**
R/R				*				*		**		*	
N/R						*	*		**	*	**	**	
R/T													
A/R									*	**	*	**	
A/N								*		**		*	
A/T								**		**			
N/T									**		**	**	
R/P										**			**
N/P											**	**	**
P/P													**

FEMALE

Genotype	N/N	T/T	R/R	N/R	R/T	A/R	A/N	A/T	N/T	R/P	N/P	P/P	A/P
A/A		**							*		*	**	
N/N		*								*		*	
T/T				**	**	**	*	**		**			**
R/R										*		*	
N/R												*	
R/T												*	
A/R												*	
A/N										*		*	
A/T												*	
N/T										**			**
R/P											**	**	
N/P													**
P/P													**

CHAPTER FIVE

CYTOLOGY

5.1 INTRODUCTION

It is known that recombination regulates the generation of genetic variability by restricting the types and frequencies of recombinants produced. In eukaryotes, recombination is a function of three main processes:

(i) the frequency of crossing over,

(ii) re-assortment of parental chromosomes, and

(iii) random combination of gametes during fertilisation which generates a large array of zygotes.

Moreover, linkage provides a subtle and powerful means of concealing and maintaining variability through its restriction on recombination. Mather (1943) in attempting to explain response patterns to artificial selection reasoned that genes were tightly linked in balanced combinations such that the major component of genetic variability was 'potential' rather than 'free' (i.e. expressed). This variability could only be released by recombination within a tightly linked group of co-adapted genes. Additionally the amount of genetic variation released through recombination is affected by the frequency and distribution of chiasmata along the chromosomes. Therefore, if the distribution is non-random and/or the frequency of crossing over is low, then recombination is restricted.

The theory that chiasmata act in a mechanical way during meiosis and that they are causally related to crossing-over (Darlington, 1932) is now widely accepted. Indeed, there is evidence that chiasmata represent the

visual expression of inter-homologue recombination (Lewis and John, 1963; John and Lewis, 1965).

Since recombination plays such an important role in the release and maintenance of variability, it is not surprising to find that various genetic mechanisms operate to control the process. Ehrlich and Holm (1963) have outlined possible genetic controls of recombination, whereas Lewis and John (1.c.) have examined the control of recombination at the cytological level. Chromosome behaviour and the position and frequency of chiasmata, being under genetic control (Rees, 1961), must also respond to the action of selection. It would therefore not be totally unexpected to find different optimal levels of chiasma frequency and position for each bivalent in different populations. This possibility was investigated in a number of populations of *Chortoicetes*. In addition, Nolte's (1962 *et seq*) hypothesis which is outlined in 5.4 was examined for this locust.

Achiasmate or non-chiasmate meiosis occurs sporadically in many invertebrate species and it is quite a common means of absolute linkage in the higher Diptera. In such cases chiasmata are not formed during meiosis and there is no crossing-over. Males of the Drosophila Genus (with the exception of *D. ananassae*) and the female silkworm (*Bombyx mori*) are two well documented cases of this phenomenon. Chiasmata are known to occur in both sexes of *Chortoicetes* so the possibility of absolute linkage for all loci may be discounted for this species.

The second means of creating a genetically differential segment, where crossing over would be a rare occurrence, is provided by structural heterozygosity. Such chromosomal mutations result from errors in chromosome duplication or in the mechanism of chromosome movement on the spindle. Either of these may give rise to three classes of numerical chromosomal mutations.

(i) Inversions result from an intra-chromosomal exchange such that the order of the genes in a chromosomal segment is reversed. Pericentric inversions include the centromere whilst paracentric inversions do not. Provided such inversions are large enough to be recognised under the light microscope they are easily revealed in the heterozygous form where one normal and one mutant homologue make up the bivalent.

(ii) Translocations move chromosome segments to another part of the same chromosome or attach a fragment to a non-homologous chromosome. The length of the segment exchanged may vary up to the maximum of an entire arm.

(iii) Centric exchanges form the third category of chromosome mutations. These involve the centromere and are of two types. Centric fission involves the lateral division of the chromosome through the centromere, whilst in centric fusion, the centromeres fuse giving rise to aneuploidy. White (1954) has indicated that centric fusions have been particularly important in altering sex determination mechanisms.

All these structural mutations alter linkage relationships, many of them restricting or canalising recombination. As well as point mutations, chromosome mutations are rare, they

influence only one member of a pair of homologues at a time.

Localised chiasma formation provides yet a third method of creating a genetically differential segment. Indeed, genotypic control of chiasma frequency in grasshoppers seems to be extensively based on localisation, and according to White (1958), very low chiasma frequencies almost always involve extensive localisation. Chiasmata are formed only in the proximal or distal region of the bivalent leaving large sections of the chromosome free from crossing over and resultant recombination. In the Acrididae and Tettigoniidae there is frequently a combination of proximal and distal localisation, there being a chiasma at each end of the acrocentric bivalents, whilst the intermediate chromosomal region has a very low chiasma frequency (White, 1958). Moreover, the chromosomes of a particular species need not show the same type of localisation, although they often show the same kind to different degrees. Thus in many grasshoppers, localisation is of the proximal-distal type in the larger bivalents, and proximal only in the smaller ones.

5.2 MATERIALS AND METHODS

A cytological examination of mitosis and meiosis was carried out in both races and the hybrids.

Embryos about five days old were removed from the investing chorion and pre-treated with 0.05 per cent colchicine in physiological saline for 1½ hours at 32°C before examination. The cytology of giant neuroblast cells

enables unequivocal identification of the centromeric position for each chromosome. Chromosomes which are genuinely two armed show clear evidence of a centromeric constriction between the two arms, whilst acrocentric chromosomes are also distinctive. Ovariole sheath tissue was also pre-treated with colchicine for one to two hours before fixation. Other females were injected through the abdomen with 0.05 ml of colchicine 24 hours prior to dissection. Colchicine acts to prevent spindle formation resulting in the accumulation of a large number of cells at the metaphase stage of division. Testes were dissected *in situ* and fixed immediately in 1:3 acetic alcohol. Squash preparations were made using lacto-propionic orcein.

The populations of *Chortoicetes* used in the chiasma frequency study were:

Eastern Race

1. Plevna - a collection of adults from a loose swarming local population near Trundle, N.S.W. 10.3.1971.
2. Hay - a collection of adults from a large incipient swarming population in the Murrumbidgee Irrigation Area, N.S.W. 15.4.1971.

Western Race

A collection of nymphs from the Perth area - collected by Dr. O.R. Byrne 15.5.1971. These locusts were collected one or two at a time across a fairly large area. Because of the scattered distribution of the locusts, they could not be said to represent any one area. Moreover, the nymphs collected ranged from first instar to fifth instar. The chiasma frequency for this culture was obtained by rearing

second instar males in isolation followed by testis dissection at fledging.

Some males from each of the eastern race populations were culled at random and fixed directly in the field to establish the field chiasma frequencies. Stocks were subsequently maintained under glass-house conditions supplemented by radiant heat and constant light from sixteen 250 watt light globes suspended above the rearing cages. During the period of study the ambient temperature of the glass-house varied from a maximum of 33^{o}C to a minimum of 25^{o}C.

Hatchings from the two eastern race populations were set up under different density regimes. The crowded condition consisted of 150 nymphs per 15.6 litre cage (cage type B) (this density corresponds to field swarming densities). Isolated animals were reared in 600 ml containers which prevented visual contact with any other locust. This, and other factors have been postulated to be important in the gregarisation process. Nolte (1962 *et seq*) has postulated that the same factors are responsible for an increase in chiasma frequency with increased gregarisation. Isolated and crowded individuals were reared at the same time to ensure similar environmental conditions. Both conditions were separated by as wide a margin as physically possible (21 feet) to limit any effect on the isolated forms by suspected pheromonal contamination from the crowded cultures. Moreover, Nolte (l.c.) has proposed that the pheromone is heavier than air so contamination should not be likely.

Each cage was cleaned and fresh wheat (also grown under glass-house conditions) was supplied daily. This

routine led to some difference in the relative humidity of the two cage types. The smaller cage type varied from about 70 per cent on addition of fresh green grass to 20 per cent immediately prior to feeding, whilst humidity in the crowded cages varied from 10 to 50 per cent. Eggs from each crowded culture gave rise to the hatchings of each successively crowded generation. Single pair matings were set up between the isolated cultures, males being removed after copulation was observed. Progeny from these matings gave rise to subsequent generations of isolated individuals. This procedure required the rearing of large numbers of individual locusts as many locusts die before reaching adulthood when reared in isolation. For each of the isolated experiments conducted, 40 nymphs were set up and less than half of these fledged in each case. Isolation was carried out on hatching to maximise the length of confinement. The number of testes collected for scoring from the isolated groups was thus smaller than ideal.

In all cases testes were removed and fixed in 1:3 acetic alcohol within 24 hours of fledging. This ensured that there were many diplotene cells, as well as eliminating possible differences in chiasma frequency due to ageing. Squash preparations were made in lacto-propionic orcein stain, using three to five follicles per preparation. Cells were examined using a 100 x oil objective and 10 x eyepieces. Mean individual chiasma frequencies were then calculated in most cases from 30 cells per individual. The only exception was the Plevna Field population where 10 cells per individual were scored. In addition to measuring

chiasma frequencies of the locusts, some measure of their phase status would have been advisable. However, as stressed in 2.3 there is no reliable measure of phase status in *Chortoicetes* so this aspect of the work was neglected.

In order to make a study of chiasma localisation, testes from 10 males of each colour pattern type from laboratory stocks of the eastern race were fixed two days after fledging. The suitability of this material for examination is emphasized in 5.3. Five cells were chosen from each individual. These were photographed and each negative was enlarged to provide a total magnification of 10,500X (similar to Southern, 1967). Bivalents were then drawn onto a sheet of white typing paper to an accuracy of about 1 mm. The distance from end to first chiasma and between successive chiasma was then measured with an opisometer, each distance being recorded.

The chromosomes are relatively large in size and offer a considerable range from the smallest to the largest. The members of the complement are usually classifiable although some difficulty was experienced with the M5 to M8 range. Fox (1973) has noted the difficulty of ranking chromosomes according to length in *Schistocerca* and it appears as if the same holds for *Chortoicetes*. If the pattern of localisation is similar in the bivalents concerned, then the interpretation remains valid. There is no significant terminalisation and the length between successive chiasmata appears to be genuinely restricted. A few first metaphase oocytes were examined and it is probable that the same factors are limiting recombination in both sexes.

Unfortunately the study was somewhat limited since it was not always possible to distinguish the centromeric position which is sometimes marked by precocious procentric condensation as in *Schistocerca* (Henderson, 1963). The problem is magnified by the telocentric nature of the chromosomes in *Chortoicetes*. Therefore, the smallest measurement obtained from end to first chiasma was recorded as the first distance. In bivalents with one chiasma, this generally corresponded to the opposite end to the centromere as is evident from the study of first metaphase cells.

Telocentric bivalents frequently have a ring configuration with a terminal distal chiasma and a proximal association which may be due merely to proximal heterochromatic segments adhering to each other, or to a genuine chiasma. In some cases the chiasma can be clearly seen close to the heterochromatic segment, but in other cases the heterochromatin obscures the details of the nearby chromatids so that it is not always possible to be certain whether a proximal chiasma is actually present or not. If identification was not possible the cell was not scored. This leads to some minor selection towards a lower chiasma frequency than is actually the case. An occasional interstitial chiasma in the long arm, always recognisable as such, gave bivalents a figure of 8 shape. Each measurement was then transferred to a computer card for analysis.

A programme was devised by Dr. A. Grassia of the Mathematical Statistics Department, C.S.I.R.O., Black Mountain, Canberra. The analysis revealed:-

1. the mean length of each bivalent, across all individuals, cells and colour patterns;
2. the mean segment lengths classified by bivalent for chiasma number and bivalent format;
3. whether there was any significant degree of localisation and, if so, whether there were any differences between the colour patterns;

It was also planned to compare across the two races by colour pattern but collection of enough material from each colour pattern of the western race was not possible until near the end of the study.

5.3 THE KARYOTYPE

The standard somatic complement of *Chortoicetes* is 2n=23 male and 2n=24 female, there being an XO/XX method of sex determination. A comparison of the chromosome sizes allows for discrimination among three different size groups. The autosomes consist of two long, six medium and three small chromosomes (Fig. 5.1). The eight largest chromosomes are all rod shaped as is common to many acridoid grasshoppers (White, 1951) and it appears as if these are telocentric supporting John and Hewitt's (1968) claim that strictly telocentric chromosomes can form part of the normal karyotype. However, White (1973) would argue that these chromosomes are in fact acrocentric.

FIGURE 5.1

Karyogram of *Chortoicetes terminifera*


X
L1
L2
Long
M3
M4
M5
M6
M7
M8
Medium
S9
S10
S11
Short

The small chromosomes of *Chortoicetes* have been examined by Hewitt and John (1971) and have been found to be exceptional in two respects.

The S9 and S10 are regularly two-armed entities, the S9 being metacentric, the S10 acrocentric. However, the S11 pair may exist in three states (Fig. 5.1).Its centromeric position varies from being a homozygous telocentric or homozygous metacentric to a heterozygous telo-metacentric combination.

As above, the centromere is telocentric in the X chromosome (Fig. 5.1). The single X of the male is positively heteropycnotic during the early stages of first prophase meiosis (Fig. 5.2). By metaphase I, the X is negatively heteropycnotic compared to the autosomes which reach their greatest degree of contraction and staining intensity at this stage (Fig. 5.2). A unique feature of *Chortoicetes* is the presence of diffential pycnosity in the X chromosome (Hewitt and John, 1.c.). Up to one quarter of the X at the non-centromeric end is more lightly stained than the rest of the chromosome (Fig. 5.2). This negative heteropycnosis differs from that shown by the whole chromosome at first metaphase. It occurs from at least zygotene to late diakinesis. Hewitt and John (1.c.) have reported that this type of behaviour is not known in any other grasshopper. The uncoiling of the X at this stage may indicate some genic activity during meiosis. Indeed, this thesis details the occurrence of the first sex linked gene ever reported in the Orthoptera (3.4). After separation of the X chromosome occurs at Anaphase II the X again exhibits positive heteropycnosity and

FIGURE 5.2

Differential Heteropycnosity of the X Chromosome (arrowed) with Respect to the Autosomes and Itself

is easily identified as a dark staining body in the spermatid nuclei which contain it. Hewitt and John (l.c.) also reported that 20 per cent of the individuals in their sample possessed B chromosomes. In the 500 or so locusts from laboratory and field samples which have been cytologically examined by me only two individuals with B chromosomes were found.

5.4 CHIASMA FREQUENCIES

The purpose of this study was to compare the chiasma frequencies of locusts collected from different populations. A further examination of the progeny (of the populations collected in the field) reared under different density regimes was also carried out as described in 5.2. The results of this work relate to a hypothesis developed by Nolte and his colleagues in a series of papers (1962, 1963, 1964a,b, 1965, 1966, 1967, 1968, 1969a,b, 1970).

In essence the hypothesis proposes a positive relationship between increased chiasma frequency and increased density. The hypothesis is associated with Uvarov's theory of phase transformation which is outlined in 2.3. Nolte suggests that a pheromone is produced in the crop of hoppers, of either phase, where it is mixed with digested food and excreted. The pheromone is olfactorily perceived and is taken into the hemolymph via the tracheal system. If the concentration of the pheromone (which is heavier than air) reaches a critical threshold (determined by the density of the hoppers), it enters the hemolymph triggering off the

melanin cycle of the tyrosine pathway by removing some inhibition of tyrosinase. Nolte proposes that some earlier metabolite of the melanin pathway is absorbed into the testis from the surrounding hemolymph where it stimulates chiasma formation. Normally, solitary locusts have relatively low chiasma frequencies and the chiasmata are often localised near the tips of the chromosomes. Thus an increase in population size sufficient to induce gregarisation would also result in an increased chiasma frequency. The subsequently increased recombination rate would then result in the breaking up of linked congeries of genes. This increased variability in such swarming populations is then postulated to be important to the locust in establishing new colonies. It is envisaged that adaptation to a new environment by a migrant swarm is attained by the action of natural selection on the new linkage groups produced by an increased recombination rate.

Figure 5.3 shows the type of cell used to assess the chiasma frequency in each population of *Chortoicetes*. Although it is not feasible to present the raw data, means and standard deviations for each experimental condition may be referred to in table 5.1. An analysis of variance (ANOVA) within each group (Table 5.2) revealed the existence of significant differences in chiasma frequency between individuals in all but one out of the 12 groups studied. This finding is in accord with that of other observations on laboratory and field populations of Orthoptera (Dearn, 1973) and indicates that the number of individuals should be

FIGURE 5.3

Sample of Cell Types Used to Assess Chiasma Frequency. Top to Bottom Chiasma Number = 18, 14.11

TABLE 5.1

Mean Individual Chiasma Frequencies and Standard Deviations by Group and Population

HAY FIELD		HAY CROWDED GENERATION I		HAY CROWDED GENERATION II		HAY ISOLATED GENERATION I		HAY ISOLATED GENERATION II	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
12.30	0.99	13.87	1.63	13.33	1.35	11.93	0.69	12.17	0.79
13.10	1.37	13.33	1.42	13.23	0.97	12.83	1.12	13.07	1.48
13.30	0.75	14.13	1.25	14.07	1.23	12.27	1.01	11.83	0.95
13.30	1.62	12.47	0.94	14.27	1.14	12.10	0.80	12.47	0.73
12.30	1.12	13.23	1.14	13.10	1.06	12.90	1.18	15.43	1.81
12.67	0.84	13.23	1.01	12.60	1.16			12.47	0.89
13.17	1.12	13.50	1.04	12.83	0.99				
12.40	1.03	12.53	0.86	13.40	0.89				
12.30	0.79	12.63	0.72	13.50	1.11				
12.87	0.73	13.37	1.40	12.67	0.88				
12.77		13.23		13.30		12.41		12.91	

PLEVNA FIELD		PLEVNA CROWDED GENERATION I		PLEVNA CROWDED GENERATION II		HAY ISOLATED GENERATION I		HAY ISOLATED GENERATION II	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
11.80	0.79	13.27	1.01	13.27	1.23	12.27	0.87	12.23	1.04
12.10	0.57	13.87	1.25	12.73	0.69	12.43	1.25	12.70	0.95
12.00	0.67	13.47	0.89	13.43	0.86	12.10	0.92	12.43	0.77
12.20	0.92	13.10	1.24	13.60	1 16	12.23	1.01	12.70	1.21
12.00	0.94	13.30	1.29	12.53	0.86	12.30	0.88	11.90	0.80
12.90	1.59	13.63	1.47	12.97	1.22	12.67	0.80		
13.10	1.10	13.40	1.10	12.87	0.86	13.07	0.91		
12.60	0.97	14.50	1.41	12.30	0.75	12.53	0.89		
12.80	0.92	13.30	1.39	13.43	1.10	12.23	0.97		
12.20	1.03	12.57	0.97			12.83	1.34		
11.60	1.08								
11.60	0.69	13.44		13.02		12.47		12.39	
12.70	1.06								
12.10	0.88								
12.40	0.84								
12.80	1.48								
12.70	0.68								
12.80	1.14								
12.60	1.65								
12.30	0.48								
12.37									

HAY DIAPAUSE		WESTERN RACE ISOLATED	
Mean	S.D.	Mean	S.D.
13.13	1.33	13.07	0.90
12.93	1.28	13.20	0.81
13.07	1.20	13.63	1.27
13.17	1.23	14.03	1.29
13.30	1.29	13.70	1.06
12.67	0.99	13.37	1.19
13.50	1.04	13.43	0.86
13.13	1.30	13.73	1.34
13.13	1.41	13.60	1.16
13.77	1.43	13.80	1.22
		13.33	1.29
13.18		14.37	1.38
		13.61	

TABLE 5.2

ANOVA within Groups for Each Treatment

Sample	Source of Variation	df	SS	MS	VR	P
Hay Field	Between Individuals	0	49.43	5.49	4.77	**
	Between cells within Individuals	290	333.70	1.15		
Hay Crowded Generation I	Between Individuals	9	82.43	9.16	6.66	**
	Between cells within Individuals	290	398.70	1.37		
Hay Crowded Generation II	Between Individuals	9	81.80	9.09	7.68	**
	Between cells within Individuals	290	343.20	1.18		
Hay Diapause	Between Individuals	9	24.15	2.68	1.69	
	Between cells within Individuals	290	460.13	1.59		
Hay Isolation Generation I	Between Individuals	4	22.89	5.72	5.96	**
	Between cells within Individuals	145	139.30	0.96		
Hay Isolation Generation II	Between Individuals	5	27.12	5.42	5.62	**
	Between cells within Individuals	174	167.83	0.96		
Western Race Isolated	Between Individuals	11	43.26	3.93	2.91	**
	Between cells within Individuals	348	470.73	1.35		
Plevna Field	Between Individuals	19	38.86	1.94	1.86	*
	Between cells within Individuals	180	187.50	1.04		
Plevna Crowded Generation I	Between Individuals	9	68.79	7.64	5.14	**
	Between cells within Individuals	290	431.13	1.49		
Plevna Crowded Generation II	Between Individuals	8	48.07	6.01	6.13	**
	Between cells within Individuals	261	255.87	0.98		
Plevna Isolation Generation I	Between Individuals	9	25.53	2.84	2.85	**
	Between cells within Individuals	290	289.13	0.99		
Plevna Isolation Generation II	Between Individuals	4	13.76	3.44	3.67	**
	Between cells within Individuals	145	136.03	0.94		

maximised at the expense of scoring a large number of cells per individual. In fact, one of the major problems in analysing chiasma frequency has been the statistical technique used to demonstrate differences or equivalence between populations and/or individuals. Nolte (1962, *et seq*) has consistently used a 'C' test although the lack of homogeneity within the observations for a particular treatment makes pooling the data and comparison of groups with a simple 'C' test invalid.

The ANOVA, the differences and the probability levels between experimental conditions for each population are shown in tables 5.3, 5.4. Considering the Hay population, the only difference is between the isolated generation one and the three laboratory crowded populations. It appears as if there is some response to the initial isolation and crowding. The chiasma frequency of the isolated group one generation was lower than that of the field and crowded groups. However, the second isolated group was not significantly different from any other group. The initial decrease in chiasma frequency was not maintained, although the mean of the isolated group two generation was still lower than that of the crowded generations. A comparison of the crowded diapause group one and crowded generation one gives some indication of the reliability of scoring. These groups were from diapause and non-diapause eggs of the Hay field stock. *A. priori*, a significant difference between the two groups would not be expected. In fact an examination of table 5.1 reveals a very small difference in the overall means of the two groups.

TABLE 5.3

ANOVA Between Groups for Each Population

Population	Source of Variation	df	SS	MS	VR
HAY	Between Groups	5	4.05764	0.81153	2.13
	Between Individuals within Groups	45	17.18423	0.38187	
PLEVNA	Between Groups	4	9.43148	2.35787	13.03 *
	Between Individuals within Groups	49	8.86740	0.18097	

TABLE 5.4

Treatment Difference and Significance Level Between Groups for each Population

HAY

		Field	Crowded Generation I	Crowded Generation II	Crowded Diapause I	Isolated Generation I	Isolated Generation II
Crowded Generation I	Difference	.458		-.071	.049	.823*	.322
	S.E. Diff.	.276		.276	.276	.338	.319
Crowded Generation II	Difference	.529			.120	.894*	.393
	S.E. Diff.	.276			.276	.338	.319
Crowded Diapause I	Difference	.409				.774*	.273
	S.E. Diff.	.276				.338	.319
Isolated Generation I	Difference	-.365					-.501
	S.E. Diff.	.338					.374
Isolated Generation II	Difference	.136					
	S.E. Diff.	.319					

PLEVNA

		Field	Crowded Generation I	Crowded Generation II	Isolated Generation I	Isolated Generation II
Crowded Generation I	Difference	-1.073***		.424*	.932***	1.046***
	S.E. Diff.	.165		.195	.190	.233
Crowded Generation II	Difference	-.649***			.508*	.622*
	S.E. Diff.	.171			.195	.237
Isolated Generation I	Difference	-.141				.114
	S.E. Diff.	.165				.233
Isolated Generation II	Difference	-.027				
	S.E. Diff.	.213				

The Plevna population presents a rather different view. Field and isolated groups are not different from each other, but all three are significantly different from the crowded groups. Indeed, the second crowded generation is significantly higher in chiasma frequency than the first crowded generation. A genuine effect of density on chiasma frequency has thus been demonstrated for this stock, the crowded groups having significantly higher chiasma frequencies than the isolated groups.

These findings present something of a quandary. The differences in chiasma frequency are certainly in the direction predicted by Nolte, but it is difficult to explain why the effect was significant in one culture and not in the other. It may be that different populations react differently to the environmental parameters affecting chiasma formation resulting in different levels of chiasma frequency.

A more detailed examination of Nolte's hypothesis has been conducted by Dearn (1973). He looked at laboratory bred stocks of *Schistocerca gregaria* and *Locusta migratoria migratorioides* which had been inbred for a number of generations. Thus, there may have been some unconscious selection such that the ability of animals to respond to changes in density by varying their chiasma frequency had been altered. In an endeavour to overcome this problem, Dearn examined a sample of field *Locusta* which showed extreme solitaria characteristics. He found that the individual chiasma frequencies of this sample were very much higher than the crowded laboratory stocks from the same area, indicating that culture conditions have probably influenced the chiasma

phenotype.

An examination of table 5.1 reveals that the highest mean chiasma frequency was recorded for the solitaria western race individuals. This supports part of Dearn's findings and argues against Nolte's idea that a low chiasma frequency is typical of a low population density. A comparison of the three field populations shows that there are significant differences in chiasma frequency between them (Table 5.5). The differences observed are in direct contradiction to those predicted by Nolte's hypothesis. Moreover, the field chiasma frequencies for Hay and Plevna are generally lower than those of the laboratory groups reared under isolated conditions (Tables 5.1, 5.4) (c.f. Dearn above) (Nolte would again predict the opposite). In conclusion, Nolte's hypothesis cannot be confirmed on the basis of the observations on *Chortoicetes*. In fact, these findings argue against the hypothesis. Dearn (l.c.) has made a critical appraisal of the hypothesis and he finds (as well as the reasons cited above) theoretical grounds for rejecting the hypothesis.

Considering the above, the amount of genetic recombination in *Chortoicetes terminifera* must necessarily be limited by virtue of the relatively low number of chiasmata per cell in the male sex. However, in some species there is no recombination in one sex but the organism still displays extensive variability from a genetic point of view. *Drosophila* is an excellent example considering the number of mutants (both spontaneous and induced) that are known (Lindsley and Grell, 1968) and the large amount of electrophoretic variation

TABLE 5.5

ANOVA, Difference and Significance Levels Between Populations

Source of Variation	df	SS	MS	VR	P
Between Populations	2	12.03731	6.01865	34.94	
Between Individuals within Populations	39	6.71819	.17226		

		Plevna Field	Western Race
Hay Field	Difference	0.406*	-0.859***
	S.E. Diff.	0.161	0.178
Plevna	Difference		-1.265***
	S.E. Diff.		.152

(Hubby and Lewontin, 1966 etc.). This is not the case in *Chortoicetes*. Hawke (1970) studied 14 enzyme systems in *Chortoicetes* using the techniques of starch and acrylamide gel electrophoresis. Seven enzyme systems were found regularly. Of 25 loci, 15 were polymorphic. Genetic evidence for the presence of two co-dominant alleles at each locus was presented. Adults of two field and two laboratory populations were screened to provide an indication of the amount of variation and degree of heterogeneity in *Chortoicetes* populations. All populations deviated from the expected Hardy-Weinberg ratios. Moreover, this was reflected in the marked deficiency of heterozygotes. Misclassification of data was ruled out as a possibility in all but the esterase system, where bands from different loci occur in close proximity. Non-random sampling was unlikely, since large numbers of locusts were collected from the natural populations and samples were screened rapidly (within one week) on return to the laboratory eliminating the possibility of differential survival of isozymic forms. No differences between the gene frequencies of males and females were found. Selection against heterozygotes at the biochemical level also appears unlikely since departure from Mendelian ratios in full sibling families would be expected (but is not found) on this basis. Inbreeding can be pin-pointed as a possible cause. Firstly, the results obtained from the natural populations are similar to those of the laboratory stocks - one laboratory stock having a long history of inbreeding (stock maintained for 17 years through several breeding 'bottlenecks'). Secondly, the ecology of *Chortoicetes* indicates the possibility

of inbreeding in some areas. One natural population was collected in a very restricted area (approximately 250 sq. yds.) there being no other locust populations within a radius of 50 miles from this isolated spot at the time of collection. This population is known to have originated from a small number of adults which had oviposited there the previous autumn. However, the second natural population was collected from a very large swarm negating inbreeding as a causal mechanism in all cases. Thus no one satisfactory explanation of the findings has been possible.

5.5 LOCALISATION OF CHIASMATA

Subjective observation of chiasma position in the chromosomes during scoring of cells for frequency suggested that localisation may be important in *Chortoicetes*. Figures 5.2 and 5.3 make it clear that the majority of bivalents in the medium and small size classes have a single distally localised chiasmata.

As outlined in 5.2 and 5.3 the excellent cytological qualities of *Chortoïcetes* have enabled a quantitative estimate of the degree of localisation in each of the 11 pairs of bivalents. Table 5.6 presents the mean total lengths for each bivalent according to the number of chiasmata. After allowing for length variation between different forms of a given chromosome with the same chiasma number there appears to be a consistent relationship between chromosome length and chiasma frequency with the qualification that

TABLE 5.6

Mean Total Length for Each Bivalent According to the Number of Chiasmata

CHROMOSOME	BIVALENT TYPE	CHIASMA NO. 1	2	3	4	5	6	7	8	9	10	11	
		CHROMOSOME TYPE											
		1	2	3	4	5	6	7	8	9	10	11	
I	$\bar{x}$	113.324	122.033	97.346	88.000	143.083	127.286	122.000	133.500	146.500		251.000	
	S.E	1.946	3.043	2.989	7.097	5.637	7.203	11.860	0.500	7.030		0.000	
	n	401	184	81	13	36	21	7	2	4		1	
II	$\bar{x}$	92.606	105.013	92.790	71.750	115.333	108.765	80.143	130.000	142.000	127.000		
	S.E	1.381	2.456	2.115	6.967	12.065	5.314	6.713	0.000	17.000	0.000		
	n	409	154	138	12	9	17	7	1	2	1		
III	$\bar{x}$	79.605	94.982	82.022	64.231	106.500	100.400	82.000	174.000	103.000			
	S.E	1.059	2.582	1.560	5.329	5.560	6.648	5.817	0.000	10.000			
	n	466	111	134	13	4	15	4	1	2			
IV	$\bar{x}$	71.431	85.000	70.891	63.853	94.000	80.625	71.400					
	S.E	0.828	2.765	1.543	3.611	3.136	4.399	4.434					
	n	515	74	110	34	4	8	5					
V	$\bar{x}$	63.320	72.707	66.521	62.065	117.000	84.000	75.000					
	S.E	0.683	2.929	1.621	2.961	28.000	8.679	14.503					
	n	575	41	94	31	2	4	3					
VI	$\bar{x}$	55.968	70.235	61.694	51.303	83.000	64.000	65.500					
	S.E	0.586	2.924	1.620	1.980	0.000	4.000	4.500					
	n	593	34	85	33	1	2	2					
VII	$\bar{x}$	50.144	55.643	54.831	45.587	122.000		49.500					
	S.E	0.518	3.032	1.761	1.746	0.000		0.866					
	n	626	14	59	46	1		4					
VIII	$\bar{x}$	43.450	52.000	48.593	40.775								
	S.E	0.436	4.546	1.656	1.031								
	n	613	7	59	71								
IX	$\bar{x}$	37.088	39.000	42.353	36.667		44.000	40.000					
	S.E	0.363	0.000	2.519	1.163		0.000	0.000					
	n	685	1	17	45		1	1					
X	$\bar{x}$	30.010		37.667	34.636			31.000					
	S.E	0.298		4.177	2.836			0.000					
	n	732	0	6	11			1					
XI	$\bar{x}$	22.279			37.000								
	S.E	0.228			0.000								
	n	749	0	0	1								
	$\bar{x}$	54.821	100.016	74.105	50.606	130.351	105.603	80.147	142.750	134.500	127.000	251.000	
	n	6364	620	783	310	57	68	34	4	8	1	1	8250

8250

the small chromosomes rarely form more than one chiasma (Table 5.7). The bottom row of table 5.6 suggests that there is a positive correlation between the absolute length of each chromosome and its chiasma frequency. There are differences between bivalents with the same number of chiasma such that  is longer than  which is in turn longer than  . The significance of these two findings is not easy to explain although Henderson (1963) reports a similar length/chiasma frequency relationship in *Schistocerca*. It may be that if a chromosome is longer and more despiralised during the early stages of chiasma formation that more chiasma can be formed with the same interference distance operating along an effectively longer chromosome. Subsequent contraction of the bivalent would then be restricted by the frequency and position of chiasmata.

In order to gain some indication of the degree of localisation the raw data was converted to $\frac{\text{segment length}}{\text{total length}}$ facilitating comparison between all bivalents by removing absolute differences in chromosome size (Table 5.8, Appendix 5.1). The information was then plotted in the form of a histogram for each bivalent (Fig. 5.4). It is immediately obvious that localisation combined with a low chiasma frequency are a feature of *Chortoicetes*. As a general rule at least 53 per cent of bivalents have only one chiasma while this figure rises to a minimum of 90 per cent for two chiasma or less (Table 5.8). The degree of localisation is indicated by the clustering of chiasma between 81 to 100 on the abscissa. The pattern is more

TABLE 5.7

ANOVA of Relationship Between Chromosome Length and Chiasma Frequency

	Total Length		Variance Ratio
A(EL B)	Chromosome	(eliminating effect due to type)	466.2829 **
B(EL A)	Type	(eliminating effect due to chromosome)	19.0798 **

TABLE 5.8

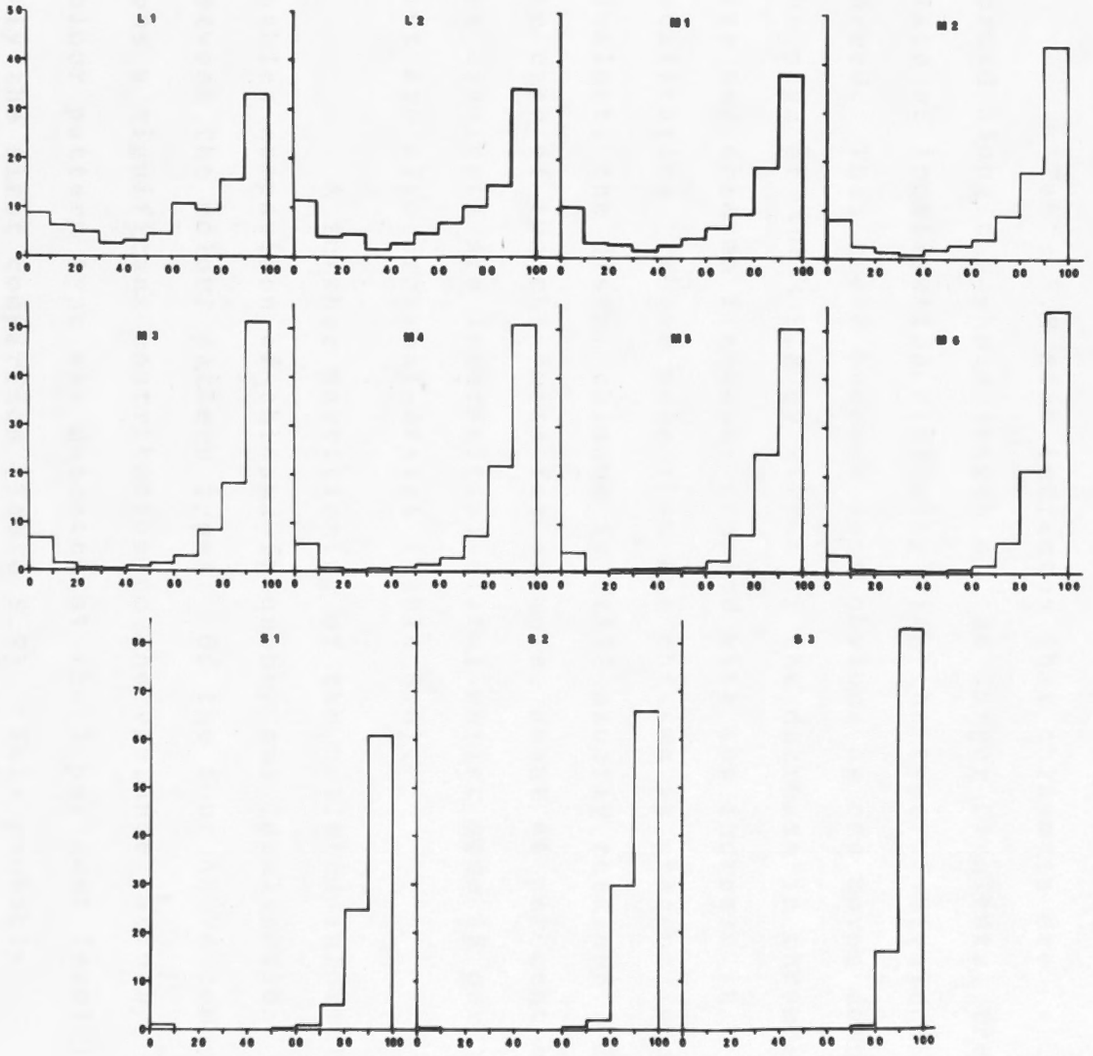
Percentage of Chiasmata Falling into the Percentage Chromosome Arm Lengths Classified by Chromosome and Bivalent Type

CHROMOSOME	BIVALENT TYPE	0 - 10	11- 20	21 -30	31 -40	41 -50	51- 60	61 -70	71- 80	81- 90	91 - 100	PERCENTAGE OF TOTAL
1	1							2.67	4.66	13.47	32.67	53
	2	1.74	5.27	3.87	1.74	1.07	2.60	4.67	2.60	0.93	0.07	25
	3	5.40	0.13	0.13	0.13	0.73	1.27	2.07	0.67	0.27		11
	5	0.53	1.00	0.67	0.40	1.00	0.60	1.13	1.20	0.67	0.27	5
	6	0.93		0.22	0.22	0.27	0.22	0.22	0.36	0.31	0.04	3
	7	0.31		0.04	0.12	0.12					0.31	1
	8,9	0.08	0.04	0.08	0.08	0.04	0.02	0.06	0.06	0.02		1
		8.99	6.44	5.01	2.69	3.23	4.71	10.82	9.49	15.67	33.36	99
2	1	0.27					0.13	1.07	5.60	13.47	33.87	55
	2	1.00	4.20	4.00	1.20	1.07	2.07	3.60	2.67	0.60	0.13	21
	3	9.20		0.13	0.20	1.47	2.47	2.33	1.93	0.67		18
	5			0.18	0.13	0.27	0.18	0.04	0.32	0.04		1
	6,7	1.07	0.22	0.27	0.27	0.31	0.18	0.13	0.18	0.13	0.36	3
		11.54	4.42	4.58	1.80	3.12	5.03	7.17	10.70	14.91	34.36	98
3	1						0.13	1.87	4.93	17.33	37.87	62
	2	0.67	3.20	2.67	0.93	1.07	1.27	2.47	1.87	0.67		15
	3	8.93		0.13	0.20	1.33	2.47	2.20	1.93	0.67		18
	5,6,7	0.93	0.09	0.13	0.40	0.49	0.09	0.18	0.40	0.18	0.18	3
	8,9	0.10			0.07	0.03	0.07	0.03	0.03	0.07		
		10.63	3.29	2.93	1.60	2.92	4.03	6.75	9.16	18.92	38.05	98
4	1						0.13	1.00	5.87	16.67	44.00	69
	2		2.47	1.60	0.47	0.60	1.13	1.67	1.20	0.73		10
	3	7.33	0.07	0.07	0.56	1.00	1.67	1.47	2.00	0.53		15
	5,6,7	0.62	0.13	0.04	0.20	0.40	0.04	0.04	0.16	0.24	0.24	2
		7.95	2.67	1.71	1.23	2.00	2.97	4.18	9.23	18.17	44.24	96
5	1	0.13						1.33	6.27	17.60	51.33	77
	2		1.67	0.73	0.27	0.40	0.27	0.73	0.80	0.53	0.07	5
	3	6.27		0.13	0.33	1.00	1.67	1.27	1.47	0.40		13
	5,6,7	0.36	0.04	0.04	0.08	0.24		0.08	0.12	0.04	0.12	1
		6.76	1.71	0.90	0.68	1.64	1.94	3.41	8.66	18.57	51.52	96
6	1	0.13						1.47	5.60	20.80	51.07	79
	2		0.87	0.80	0.33	0.27	0.47	0.80	0.60	0.33	0.07	5
	3	5.67	0.07	0.07	0.56	0.93	1.13	0.93	1.27	0.73		11
	5,6,7	0.16	0.08	0.04	0.04	0.04	0.08	0.04	0.04		0.08	1
		5.96	1.02	0.91	0.93	1.24	1.86	3.24	7.51	21.86	51.22	96
7	1	0.40					0.27	1.07	6.80	24.40	50.53	83
	2		0.20	0.53	0.07	0.07	0.35	0.35	0.28	0.07		2
	3	3.93			0.47	0.53	0.80	0.87	0.93	0.33		8
		4.33	0.20	0.53	0.54	0.60	1.42	2.29	8.01	24.80	50.53	93
8	1		0.93				0.13	0.29	5.47	21.33	54.27	82
	2		0.20	0.20	0.07			0.20	0.20	0.07		1
	3	3.93		0.07	0.28	0.28	0.73	1.20	0.93	0.40	0.07	8
		3.93	1.13	0.27	0.35	0.28	0.86	1.79	6.60	21.80	54.34	91
9	1							0.67	5.20	24.67	60.80	91
	2			0.07			0.07					
	3	1.13				0.07	0.28	0.47	0.20	0.14		2
		1.13		0.07		0.07	0.35	1.14	5.40	24.81	60.80	93
10	1							0.40	1.87	29.47	65.87	98
	3	0.40				0.14		0.14	0.07	0.07		1
		0.40				0.14		0.54	1.94	29.54	65.87	99
11	1			0.13				0.13	0.53	15.73	80.33	

FIGURE 5.4

Plot of Chiasma Distribution for each Bivalent of *Chortoicetes*

The vertical axis represents the number of observations falling within the percentage chromosome arm length at diplotene (abscissa)


L 1
L 2
M 1
M 2
M 3
M 4
M 5
M 6
S 1
S 2
S 3

pronounced in the smaller chromosomes although it is generally consistent for all bivalents. When one chiasma is present it tends to be formed toward one end of the bivalent and this is reflected by the histogram. In fact these chiasmata are distal in position, i.e. the longest section of the bivalent contains the centromere.

Table 5.8 also indicates that chiasmata are formed along the whole length of the larger bivalents, the place of localisation differing with the type of bivalent formed. This trend becomes less obvious as one moves down the page of table 5.8 by virtue of the decrease in chromosome size and chiasma frequency coupled with the increase in localisation. Where more than one chiasma is present in a bivalent, the distal chiasma is still usually retained. In the case of two chiasmata for example, about 46 per cent of the bivalents are interstitial-distal whilst some 18 per cent are also proximal-distal (Table 5.6).

A further partitioning of the collated information enables comparison of chiasma frequency and localisation between the colour pattern types. Of the four ANOVA comparisons a significant contribution to the variance ratio by colour pattern type was detected at the 1 per cent level in only the first comparison (Table 5.9). This probably reflects the biased contribution to the variance ratio by the type four bivalent. It may generally be stated that there is no significant difference in the degree of localisation between the colour pattern types, but there are differences attributable to the bivalent types. An ANOVA of the chiasma frequencies per cell for each of the colour pattern types based on the means and standard deviations of table 5.10

TABLE 5.9

ANOVA Comparing the Degree of Chiasma Localisation Among the Colour Patterns

	Source of Variation	DF	Variance Ratio
Seg $\frac{1}{T}$	Colour Pattern	14 x 14	8.6339 ***
	Bivalent Type	10 x 10	4385.4219 ***
Seg $\frac{2}{T}$	"	"	1.5546
	"	"	315.5780 ***
Seg $\frac{3}{T}$	"	"	3.0325 *
	"	"	299.7446 ***
Seg $\frac{4}{T}$	"	"	1.3878
	"	"	75.6233 ***

TABLE 5.10

Mean Colour Pattern Chiasma Frequencies and Standard Deviations

COLOUR PATTERN GENOTYPE	MEAN	S.D.
A/A	13.7600	1.5980
A/N	14.4200	2.4251
A/R	13.9200	2.0288
A/T	13.9200	1.6393
N/N	13.0000	1.1429
N/R	14.4200	1.6424
N/T	14.2800	1.8521
R/R	13.7400	1.4401
R/T	14.3200	2.4196
T/T	13.8600	1.6904
A/P	13.3200	1.3915
N/P	12.9200	1.6393
R/P	13.2400	1.2707
T/P	13.7800	1.5022
P/P	13.4600	2.0224
	13.7573	

revealed no overall differences in chiasma frequency between the ten main colour pattern types (Table 5.11). The variance ratio is significant at the 5 per cent level, but this is due solely to the nigrovirgata colour pattern. This colour pattern has a significantly lower chiasma frequency when compared with other common colour patterns (Table 5.10). There would appear to be no reasonable explanation for this finding and it is probable that this represents a pure chance effect or is attributable to experimental error.

Addition of the porphyrica classes to the analyses resulted in a very significant variance ratio (Table 5.11); examination of table 5.10 indicates that these colour patterns generally have lower chiasma frequencies than the other colour patterns. This is probably due to the fact that owing to the rarity of this type in the field (Table 3.8) the laboratory stocks have been bred from a small number of individuals collected from the field. Thus further evidence of some type of selection acting in laboratory populations to reduce chiasma frequency as discussed earlier is provided. In this case, it may be inbreeding plus the founder effect which has led to the observed differences.

In conclusion genetic recombination (as measured by the frequency and distribution of chiasmata) in *Chortoicetes* must be very limited. The number of chiasmata per cell in the male is usually slightly higher than the number of bivalents. Moreover, the chiasmata are found at localised

TABLE 5.11

ANOVA of Chiasma Frequency Between Colour Patterns

1. (Without Porphyrica Classes 11-15 of Table 5.13)

Source of Variation	DF	SS	MS	VR	P
Between colour patterns	9	83.9120	9.3236	2.79	*
Error†	490	1639.4400	3.3458		

2. All Colour Patterns

Source of Variation	DF	SS	MS	VR	P
Between colour patterns	14	167.7147	11.9796	3.90	***
Error†	735	2256.1200	3.0696		

† Error component estimated from variation between individuals and within cells from each individual for each colour pattern.

points on the bivalents. This suggests that the species may be leaning heavily on heterozygosity at many loci associated with adaptive polygenic systems which are protected from disruption by recombination. Where chiasma formation is localised, recombination occurring among the genetic loci in such regions may be of considerable importance to the species in enabling it to handle short term environmental changes. Apart from this, large sections of the genome are transferred from one generation to the next in a relatively intact state with little recombination taking place. Species are thought to have an optimum rate of recombination which represents a compromise between such requirements as adjustability to long term changes and the ability to provide some variety among individuals in each population for handling short term fluctuations in the environment (Mather, 1943). Observed recombination rates are probably not far from this optimum and there may be different optimal recombination rates in different parts of the genome.

A number of population geneticists have argued that chiasma localisation must be the result of selection for linkage. For example, Turner (1967a) reasons that under conditions of random mating and constant selective pressures, polymorphic populations with linkage disequilibrium are always subject to epistatic selection for reduced recombination between the relevant loci. Under such conditions, co-adapted gene complexes will evolve, characterised by the possession of large linkage groups within which there is little recombination.

Most would accept that the primary reason for the retention of chiasmata in the meiotic mechanism of most organisms is the mechanical one, i.e. organisms are subject to a very rigid system of canalisation whereby each bivalent possesses at least one chiasma which is of fundamental importance in a mechanical way ensuring regularity of disjunction (Darlington, 1932). The secondary role of chiasmata is the genetic one *viz* the role in recombination. Selection must have acted to ensure that the mechanical and genetic functions of chiasmata in *Chortoicetes* interact in such a way to permit the necessary recombination for evolution.

CHAPTER SIX

GENERAL DISCUSSION

Whenever the environment so changes that a rare major gene (*viz*, one having an easily detectable effect) begins to spread, it will usually result in a polymorphic situation maintained by contending advantages and disadvantages. Genetic polymorphism then, is a type of variation in which individuals possess discrete characteristics yet co-exist in the one population. It is defined as 'the occurrence together in the same habitat of two or more discontinuous forms or phases of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation' (Ford, 1940). If there was no balance of selective forces one variety would be expected to increase in frequency, to the exclusion of the others, since it is unlikely that different varieties should retain the same fitness in the same environment (Fisher, 1930a). Thus polymorphic phases must be very common and unless they are undergoing a period of replacement whereby a new advantageous mutant is replacing its allelomorph to become the new wild type (transient polymorphism - e.g. industrial melanism), they will be balanced at neutrality by opposing selective forces. However, Fisher (1930a) has pointed out that the range of conditions over which such neutrality could exist must be very small.

Mather (1953) classified selection into three basic types. It may -

(i) favour one extreme phenotype at the expense of all others as in artificial selection (directional selection),

(ii) favour the average expression at the expense of both extremes (stabilising selection), or

(iii) favour both extremes simultaneously, though not necessarily to the same extent at the expense of the average (disruptive selection).

Disruptive selection may also lead to speciation if the optimal phenotypes are functionally unrelated to one another, or to polymorphism if they are related.

Thoday and his colleagues have established that polymorphism for bristle number may be brought about in *Drosophila melanogaster* by disruptive selection (Thoday and Boam, 1959, 1961; Thoday and Gibson, 1962). The discontinuous phenotypes that occur in mimetic and sexual polymorphism may also be attributed to disruptive selection. Indeed, most cases of phenotypic polymorphism that have been reported recently are claimed to be due to the result of disruptive selection. However, it is difficult to envisage how such selection could apply to blood groups or banding patterns in snails. Generally, phenotypic polymorphism seems to be maintained because it contributes directly to fitness or usually because the same gene has physiological side effects that contribute to fitness.

It was at one time supposed that net fitness could be measured directly, by comparing the frequencies of genotypes at any point in the reproductive cycle with the Hardy-Weinberg frequencies predicted from the genotypes in the previous generation at the same point of the cycle. In a fundamental paper on fitness estimation Prout(1965) showed that this

method is erroneous and does not take proper account of differential fertility. The most direct approach of assessing selection then is to measure the reproduction of the various genotypes at a locus and calculate fitness values. Although there is no difficulty in theory in estimating fitness, in practice the difficulties are virtually insurmountable. For populations with overlapping generations such as *Chortoicetes*, the age-specific mortality and fecundity schedules for each genotype need to be measured, and these must be combined in a special way to give fitness estimates (Bodmer, 1968; Charlesworth, 1970; Charlesworth and Giesel, 1972).

Even though net fitness cannot be measured in nature, individual components can be, providing a partial picture. Thus, if alleles are segregating at intermediate frequencies and any selection at all can be demonstrated to operate on them, it would be difficult to avoid the conclusion that they are held by some form of balancing selection (Lewontin, 1974).

It cannot be categorically concluded from the experimental work of this thesis whether the colour pattern polymorphism is transient or balanced as data from a number of successive generations are necessary to decide this point. However, it does seem likely that the polymorphism is of the balanced type. There are no monomorphic populations and specific gene frequencies are not found to be typical of different geographical regions. For multiple alleles in a selectively balanced polymorphism, the necessary conditions for a stable equilibrium would require that no heterozygote be less fit than both the associated homozygotes (Owen, 1953;

Kimura, 1956; Mandel 1959). In both natural and laboratory populations of *Chortoicetes* the heterozygous colour patterns have been shown to be more fit than their associated homozygotes (4.5b, 4.6, 4.7)fulfilling the requirements. However, the equilibrium conditions assume random mating and constant selective values. In the latter case, the homogeneity among samples of colour pattern genotypes collected by Byrne (1967b) indicates that there were no real differences in the rate of change in genotype frequency over all samples. One can conclude from this that the relative selective values were fairly constant and were the same for the different stages of development. In regard to random mating the evidence presented in 3.5 and 4.3 suggests that the populations of *Chortoicetes* are panmictic.

It has been established in 4.6 that the viability of the homozygous colour patterns of *Chortoicetes* is about 83 per cent of that of the heterozygotes in the eastern race. In the western race the figure is 96 per cent although the number that the estimate is based on is smaller than in the eastern race (1147 nymphs versus 2930). Moreover, it was possible to group the colour patterns in successive order of viability in the eastern race such that N/R, R/T were more viable at the end of first instar over N/T, A/T, A/N over N/N, A/A, T/T over R/R. The difference in culture was such that the 'single' heterozygotes (N/R, R/T) of the eastern race enjoyed:

(i) a 6 per cent advantage over double dominant heterozygotes (N/T, A/T, A/N),

(ii) a 19 per cent advantage over dominant homozygotes (N/N, T/T, A/A), and

(iii) a 20 per cent advantage over the 'universal recessive' R/R.

Such values as above if present in the wild would be sufficient to ensure a stable polymorphism through the advantage of the heterozygous over the homozygous forms.

Table 6.1 shows the percentage of the genes for different dominants which in the following generation would have been in double dominant combination. Since 41.34 per cent of the eastern race and 46.36 per cent of the bearers of these genes in the western race are eliminated (4.5b), the loss is:

(i) eastern race - from 0.01 to 3.15 per cent

(ii) western race - from 3.49 to 3.58 per cent.

In this sense of powerful selection, other factors may also be eliminated when crossing over does take place. Thus by the differential elimination of colour pattern types via linkage disequilibrium most of the unwanted types resulting from recombination would also be eliminated.

In assessing the effect of laboratory conditions on viability from first instar to fledging the optimal rearing (wet food, 15½ hours photophase, $33^{o}/27^{o}$ - q.v. 4.7) condition in the eastern race favoured a similar grouping. R/R, R/T, N/R enjoyed an advantage over N/N, T/T which in turn were more viable than A/A, A/T, N/T. The number of hatchlings was also determined for each colour pattern indicating a greater number of hatchlings for R/R over N/T, A/N, A/T over R/T, A/R, N/R over N/N, T/T, A/A. The amount of data available for the western race limits the value of comparison,

TABLE 6.1

Percentage of Double Heterozygotes Found in Each Generation

(Calculated from the mean gene frequencies of Table 4.9)

Population	A/N	N/T	A/T
Hay Spring 1971	0.04	3.42	0.03
Hay Autumn 1972	0.08	1.00	0.05
Boulia Autumn 1972	0.31	7.63	0.31
Coonamble Spring 1960	0.22	5.47	0.31
Trangie Autumn 1973	0.16	2.04	0.21
Coona Autumn 1973	0.05	1.17	0.18
White Cliffs Autumn 1973	0.04	0.88	0.10
White Cliffs Spring 1973	0.29	2.20	0.40
Tibooburra Spring 1973	0.18	2.46	0.25
Western Race 1961		8.06	
Western Race 1971/72		8.26	

although where sufficient data is available it generally parallels that of the eastern race. A more sophisticated analytical method of measuring fitness along the lines of that developed by Prout (1971a,b) would provide useful additional information. This would involve setting up contrived numerical mixtures of genotypes as adults and recording the proportion of genotypes among the offspring. Fitness estimates are then calculated for the sexes from the values with which the known proportions among the parents must be weighted in order to account for their offspring.

Although it is not possible to infer the magnitude of net heterosis from the measurement of partial fitnesses since there is no necessary correlation between any one component of fitness and total fitness except insofar as parts are correlated with wholes, the rubiginosa pattern provides an interesting example of opposite fitness relations in different parts of the life cycle. Thus it has been demonstrated that the rubiginosa phenotype suffers a 20 per cent disadvantage in culture between hatching and first instar when compared with single heterozygotes (N/R, R/T) of the eastern race (4.6). From first instar to fledging rubiginosa enjoys an advantage over the other colour patterns except N/R, R/T (4.7). The initial disadvantage must also be offset to some extent by the fact that the rubiginosa types produce a significantly larger number of hatchlings than do the other pattern types (4.6).

It follows that selective intensities in favour of the heterozygotes must be balanced by selective elimination of the homozygotes in order to give stability to the proportions of competing dominants. The elimination of double

heterozygotes sufficiently explains the absence of chromosomes with two dominants in coupling, for these would be subject to intense elimination generation after generation, whereas the doubles in repulsion will be made up afresh by singly dominant parents. The values above are of necessity averages but they reflect a complex system of selection which has many implications for the organism, among these being the phenomenon known as genetic load.

As well as colour pattern polymorphisms the development of electrophoretic techniques has led to the discovery of many enzyme polymorphisms in which two or more alleles, at a locus, are maintained at a high frequency. Such polymorphic loci differ from the usual type of locus, not in the number of different phenotypes which are produced but in their frequency. The extent of such heterozygosity in natural populations has been estimated by Lewontin and Hubby (1966) who studied a number of proteins in *Drosophila pseudo-obscura*, and concluded that the average individual is heterozygous for at least 12 per cent of genes in the entire genome. This value is comparable with that found by Harris (1966) for man, using enzymes of the blood. O'Brien and MacIntyre (1969) using *Drosophila melanogaster* arrived at a figure of 22 per cent whilst Selander and Yang (1969) using *Mus musculus* obtained a value of 11 per cent. These loci are presumed to be maintained by some form of "balancing selection" which involves opposing selection forces. When an allele selected against in the homozygous state is retained

because of the superiority of heterozygotes (overdominance), a balanced equilibrium results. The possibility that the high level of heterozygosity was attributable to heterozygote advantage, was also examined by Lewontin and Hubby (l.c) and they considered the explanation suspect, on the grounds that such a high level of heterozygosity would imply an enormous genetic load on the population as postulated by Kimura and Crow (1964).

Objections about the genetic load argument based on theoretical (Sved *et al*, 1967) and ecological considerations (Wallace, 1968, 1970) have been raised. Franklin and Lewontin (1970) using a multi-locus system where linkage is also considered have shown that the upper limit on the number of polymorphisms which may be maintained by heterosis is much higher than that postulated by Sved *et al*, (l.c.) and that heterozygosity may be due to heterozygote advantage without placing an excessive genetic load on the population. Nevertheless, some authors have contended that either selection coefficients for polymorphic loci are vanishingly small (c.f. those found in this thesis), or structural variants are adaptively neutral with respect to natural selection (Kimura, 1968; King and Jukes, 1969). This notion of adaptively neutral isoalleles has been termed non-Darwinian evolution. Clarke (1970) and Richmond (1970) reviewed the evidence and both concluded that the genetic variation observed in populations is most compatible with a neo-Darwinian interpretation. Their conclusion is supported by population studies which have demonstrated the effects of selection on genes controlling the protein structure of

isozymes (Kojima and Tobari, 1969; Koehn, 1969; Gaines and Krebs, 1971).

Although the fecundities, fertilities, viabilities and other components of fitness of field populations are almost certainly different from the laboratory situation, perhaps the most important feature of Chapter 4 is the demonstration of differential fitnesses between colour patterns reared under the same and different environmental conditions (4.7). Fisher (1930a) has pointed out that the most general basis for maintaining polymorphism is that in which the heterozygote is at an advantage compared to both homozygotes and this appears to be the case in *Chortoicetes*.

In the wild then, a balance of different forms in different populations is probably maintained by the physiological advantage of certain gene combinations, the actual ratios of genotypes being altered in different directions by the action of natural selection. The significant difference in gene frequencies between the natural populations indicates that some type of (unidentified) ecological selection must be operating in different directions determining the actual ratios of the genotypes in different areas (4.2b). However, there does appear to be some type of balanced cyclical selection acting particularly on the nigrovirgata and rubiginosa genes in a complementary way but in opposite directions such that they are favoured during one part of the year and selected against during the other part (4.4).

To explain the fluctuations one only has to bear in mind that the autumn and spring populations are separated by at least five months during the over-wintering of eggs. The ecological characteristics of the habitats at these two times is different, particularly with regard to temperature, humidity and vegetation. As the season advances from spring to summer the habitat becomes progressively drier and its background colour becomes relatively more uniform. The rubiginosa colour pattern is apparently better adapted than other colour patterns to this kind of environment. From the laboratory experiment described in 4.7 it does not seem that rubiginosa individuals are physiologically better adapted to withstand dry conditions than most other genotypes. However, measurements of body temperature of locusts using microelectrodes inserted into the hemolymph have revealed differences between the colour pattern types which have not been quantified as yet (Hawke and Aston, unpublished). It may be that rubiginosa individuals are able to utilise and retain the available moisture more efficiently during oppressively hot conditions.

The rubiginosa pattern which is uniformly brown with black mottling (Fig's 3.1, 3.2) certainly appears to be less prominent than other colour patterns when on backgrounds such as dead plants and exposed areas of soil. It would therefore be likely to have a selective advantage in such an environment against the action of predators. Conversely, this pattern is very conspicuous against the lush green conditions which usually occur towards autumn and early

spring where it seems to be at a disadvantage. So far, no data has been obtained on the effect of predation on colour pattern frequencies although the work of Carrick (1959) on the food and feeding habits of two species of ibis (*Thresikornis spinicollis* and *T. molluca*) suggests that these birds may be important predators of *Chortoicetes*. Other predators of *Chortoicetes* which are commonly encountered in the field include spiders, Psammocharid wasps and Asilid flies.

It must be remembered that the visible phenotype is only a limited part of the manifestations of the genotype. Colour patterns are an end product of the complex process of differentiation and it is unlikely that genes which affect the physiology of an organism should have no effect on viability. In fact major genes always have multiple effects and their mutation occurs at random relative to the needs of the organism. Moreover, major genes often affect the characters which are of greatest evolutionary importance, such as fertility, fecundity, sexual vigour, longevity and ability to withstand environmental extremes. Consequently, if one of the features for which a mutant is responsible gives it an advantage, others are very likely not to do so and they will almost certainly be deleterious. As Sheppard (1953) has pointed out, selection will tend to make the beneficial effects dominant and the harmful ones recessive. In these circumstances, the homozygote will, relative to the alleles in question, have both advantages and disadvantages, whilst the heterozygote will tend to have advantages only, ensuring its superiority over the other two genotypes thus establishing a basis for polymorphism. This situation is

illustrated in the well studied organisms such as *Drosophila* and *Ephestia*. In *Drosophila* nearly every known mutation affects one or other component of fitness and usually several simultaneously (e.g. see Lindsley and Grell, 1968). Caspari (1950) working with *Ephestia kuhniella* showed that the alleles responsible for the production of red or brown testis colour also have an effect on a number of other features. Some of these are advantageous and dominant whilst others are deleterious and recessive.

The fact that the majority of mutants are phenotypically recessive to the normal wild type led Fisher (1928a,b; 1929; 1930a) to formulate his theory on the evolution of dominance. He suggested that, on its first appearance, a mutant gene usually produces a marked effect, and it only becomes recessive to the wild type as a result of selection acting over very long periods of time. Fisher's theory regarded this process as being due to the selection of specific modifying genes which render the appearance and viability of the heterozygote approximately the same as those of the wild type. In this way it is possible to argue that the dominance exhibited by wild type alleles has been achieved by the accumulation of genetic modifiers resulting from selection acting on heterozygotes.

Fisher's theory evoked considerable criticism notably by Wright (1929 a,b; 1934a,b) who subsequently developed his own theory. Other alternatives were proposed

by Haldane (1930), Muller (1943) and Plunkett (1933). None of the theories are entirely satisfactory and it is probable that dominance may not always evolve in the same way, each hypothesis providing the correct explanation in different cases. A review of the major theories is contained in Sheppard (1967) and Wallace (1968), whilst the historical background and a resume of some of the experimental evidence related to the theories of dominance, is contained in Sved and Mayo (1970). The resurgence of interest in this problem began with Crosby (1963) who questioned the validity of all theories. Recent developments in the field have been summarised by Murray (1972). Detailed mathematical analyses have also been carried out, often with contradictory results. These arguments are almost always related to Fisher's model, as the other theories are not easily amenable to mathematics and computer simulation studies. Most mathematical studies have involved a 'one major gene - one modifying locus' model which certainly oversimplifies the natural situation. Problems of definition, a major stumbling block, need to be resolved before too much credence can be attributed to this sort of analysis.

Both Fisher (1930a,b) and Haldane (1930) emphasized the importance of polymorphism in the theory of dominance modification. Polymorphism necessitates the production of large numbers of heterozygotes thus overcoming the principal objection (Wright, l.c.) to Fisher's theory.

Haldane (1930) called attention to the fact that in certain organisms, the mutant genes are generally dominant and not recessive to the normal wild type maintaining that

the facts where not in agreement with Fisher's (1928a) theory of dominance. For example, in *Primula sinensis*, eight out of the 34 genes studied were incompletely recessive (Winton and Haldane, 1933). A number of other diverse organisms also exhibit polymorphism in which rare genes are dominant to their more common alleles. Nabours' (1925, 1933, 1950) classic work with the grouse locusts revealed 30 elementary patterns in *Paratettix texanus*, 15 in *Apotettix eurycephalus*, over 25 in *Acrydium arenosum* and *Acrydium granulatus* and several in *Tettigidea parvipennis pennata* and *Telmatettix aztecus*. These elementary patterns, amounting to over 100 in the various species, are with two or three exceptions dominant in inheritance. Similarly, Winge (1927) found 18 genes dominant to the normal and none recessive in the fish *Lebistes reticulatus*. Other cases, less completely studied, also fall into the same category (Nabours, l.c.). In answering Haldane's criticisms Fisher (1930b) demonstrated that the data were not inconsistent with his hypothesis and pointed out that the land snails, *Cepaea hortensis* and *Cepaea nemoralis* could be added to the list. The inheritance of the colour patterns in *Chortoicetes terminifera* suggests that it should also be placed in this group.

Most of the species cited above, though belonging to different phyla, have a number of remarkable characteristics in common. The wild forms are visibly polymorphic and in each case the polymorphism is determined by genes or gene-complexes which are probably very closely

linked in inheritance so that they act as a 'super-gene'. Each has a number of colour pattern morphs, which if allelic, have heterozygotes combining the characteristics of the two dominant homozygotes. In striking contrast to the mimetic Lepidoptera, the polymorphisms appear to have no superficially obvious adaptive significance. Where analysis has been possible the number of linkage groups has been found to be smaller than the number of chromosomes. For example, *Apotettix* has one autosomal linkage group with seven pairs of chromosomes, *Paratettix* three linkage groups with seven pairs of chromosomes and *Lebistes* has 23 pairs of chromosomes with 30 autosomal genes and the other 282 sex-linked(Haldane, 1930; Haskins *et al*, 1961). Additionally, most species have a relatively common form (termed the 'universal recessive') which is recessive to all other forms. Why the most common form is so often the universal recessive is unclear. Since morph genes are probably selected in most cases for their physiological effect, the recessive gene for the morphological contribution may well be dominant with respect to its physiological phenotype. Such dominance would permit the rapid spreading of a morphologically recessive gene. Fisher (1930b) suggested that the advantage of the single dominant heterozygous forms was probably opposed by counterselection against the homozygotes curtailing the spread of the dominant genes. Indeed, in the grouse locusts (Fisher, 1930b; 1939) and *Chortoicetes* (4.5b) the situation is probably parallel where the dominant genes are advantageous by themselves (single heterozygotes) and disadvantageous when they interact (double dominant heterozygotes, homozygotes).

The 'super-gene' concept was introduced by Darlington and Mather (1949) as 'a group of genes acting as a mechanical unit in particular allelic combinations'. Members of the group, though consisting of major genes are so seldom separated by crossing over that they operate as a single genetic entity (i.e. multiple alleles). It seems clear then, that in the majority of instances at least, the genetic control and interaction of co-adapted characters such as colour patterns must be vested in separate genes which have been collected together to form a switch unit. Chromosomal re-arrangements transferring the genes to the same chromosome would then be favoured. Subsequently, selection could operate to reduce crossing over between them, as it would if they chanced to be linked in the first place. This end may be achieved in several ways.

Individual major genes are nearly always pleiotropic in nature - they influence the general physiology of the organism owing to some change in enzyme production, even if the visible structures which they control are quite trivial. Yet it is extremely unlikely that the multiple action of a single gene can often lead to the production of the diverse co-adapted characters required in a polymorphism. A similar improbability arises if it is supposed that a super gene is built up by duplication or the breakage of a cistronic unit. Haldane (1930) has suggested that it is due to the translocation of segments from one chromosome to another. The unusual phenomenon of the dominance of the mutant types over

the 'universal recessive' could then be accounted for, since the mutants would presumably possess the translocated segment in duplicate, both in its original and new position (Huxley, 1942). Reconstruction of the chromosome could then result in the genes being moved closer together forming a tighter linkage group.

The hypothesis that there will be selection for increased linkage between genes which interact with respect to their selective advantages was first put forward by Fisher (1930a) and has been discussed with possible examples by Sheppard (1953). As crossing-over decreases between linked loci controlling a polymorphism, a smaller selection pressure suffices to maintain the combination of alleles most advantageous to each phase, or it does so more effectively. When the loci are so close together that a cross-over between them is extremely uncommon, a super-gene is formed and the effect will simulate that of multiple allelism. Unless a cytologically recognisable inversion is involved the distinction between multiple allelism and close linkage can only be made when a rare chiasma occurs at the right place. Turner (1967a,b) has considered the theoretical aspects of the evolution of super genes and has shown that epistatic selection and balanced polymorphism tend to produce super-genes, other forces preventing the process from leading to total condensation of the genome.

An alternative to the translocation hypothesis is that selective forces are such that only appropriate mutations

which occur on the relevant chromosome can be preserved and incorporated into a super-gene (Kimura, 1956; Lewontin and Kojima, 1960; Haldane, 1962). Whichever alternative is preferred (presumably sometimes one, sometimes the other), Ford (1971) stresses that both of them favour few large chromosomes. Thus considering the frequency and importance of polymorphism, the presence of super-genes must be one of the factors limiting increase in the number of chromosomes.

The relatively small number of chromosomes and the evolutionary effect of reduced cross-over values result in several implications which warrant further consideration. Chromosomal observations have shown that there is no unconditional optimum chiasma frequency (Darlington, 1932). Moreover, it is an often neglected fact that chiasmata serve two qualitatively different purposes. Most cytologists assume that a chiasma is the physical manifestation of a cross-over event and that the number of chiasmata is correlated with the amount of genetic recombination. The other important function of chiasma formation is to ensure that the main sequence of events in meiosis can occur - i.e. pairing, reduction etc., which constitute the internal mechanism of sexual reproduction. The meiotic process usually demands the formation of at least one chiasma per bivalent for orderly separation of the chromosomes.

The first condition of chiasma frequency being indicative of cross-over frequency places the upper limit on chiasma formation, whilst the second condition (chiasma as a structural entity) may impose a lower limit on the

frequency of crossing-over. Two modes of controlling recombination are possible: structural control by inversions, or interchanges which results in suppression of recombination within the inverted segment such that the material is inherited as a unit. Reference is made to the part which inversions play in restricting recombination on page 98 along with other strategies for producing genetically differential segments. It should be noted that inversions in grasshoppers do not generally behave in the same manner as those of the Diptera. In fact all the inversions which have been detected are pericentric, there being no evidence for paracentric inversions in grasshoppers. (White, 1973). Short inversions which are difficult to detect have been found in some grasshoppers (White and Morley, 1955) and they may disturb chiasma formation and provide the basis for super-gene formation by maintaining small numbers of loci in an intact state. Genotypic control is also known through the agents of cross-over inhibitors which suppress crossing-over, and localised chiasma formation which limits recombination to particular regions of the chromosome complement and genome.

In *Chortoicetes* the number of chiasmata per cell in the male is slightly higher than the number of bivalents (Chapter 5). Moreover, chiasmata are found at localised points on the bivalents, restricting recombination even further. If the same situation exists in the female, the amount of genetic recombination for the whole of the genome must be very limited for this locust.

Fisher (1930a; 1931) suggested on theoretical grounds that the mean fitness of a polymorphic population is increased by a decrease in the frequency of recombination. Turner (1967a) asserted that this was not always the case. However, exact treatment of specific simplified models considering two gene systems (Kimura, 1956; Lewontin and Kojima, 1960; Lewontin, 1964; Karlin and Feldman, 1969) and more complex methods treated by numerical methods (Lewontin, 1964, 1971; Franklin and Lewontin, 1970) have confirmed Fisher's conjecture. Thus 'a population held in a stable polymorphic equilibrium by natural selection has a mean fitness at equilibrium that generally increases with tighter linkage' (Lewontin, 1971).

Because of the intense selection against double dominant heterozygotes, homozygous dominants and the 'universal recessive' it follows that there would be strong selection in favour of any mechanism(s) suppressing crossing-over between the loci determining the polymorphism. Considering the implications of the previous pages, the number of chromosomes of polymorphic species would be expected to be rather low and other factors operating to inhibit crossing-over may also be present. In fact, an examination of table 6.2 reveals that this hypothesis is generally supported amongst the organisms cited in this Chapter. Haldane (1920) has suggested that in the grouse locusts (and Demerec(1928) with respect to *Lebistes*) the linkage and apparent allelomorphism observed was due not only to the infrequency of crossing-over in the chromosomes but to the fact that there was linkage between chromosomes

TABLE 6.2

Chromosome Numbers of Different Species

Organism	Chromosome Complement	Comment	Reference
Chortoicetes	22A XO Male XX Female	Low chiasma frequency. Localised chiasmata.	
Cepaea nemoralis	22	Low chiasma frequency. Localisation with respect to A group bivalent.	Bantock, 1972
Lebistes reticulatus	22A XY Male XX Female	Colour pattern genes restricted to sex chromosomes.	Haskins *et al* 1961
Tettigidae Paratettix texanus	12A XO Male XX Female	Crossing-over in both sexes.	Nabours, 1929 *et seq.*
Tettigidea parvipennis pennata	12A XP Male XX Female		"
Acrydium arenosum	12A XO Male XX Female		"
Telmatettix aztecus	12A XO Male XX Female		"
Apotettix eurycephalus	12A XO Male XX Female	Crossing over almost exclusive to the female	"

so that several chromosomes were transmitted as a group from parent to offspring. Haldane's (1930) study of Nabours' (1925) linkage map for *Apotettix* suggested the possibility that 11 genes lie in four or five of the seven pairs of chromosomes and that crossing-over within a chromosome was rare. He cited the cytological facts known in other Orthoptera, notably the work of Carothers (1917, 1921) as support for his view. Darlington (1930) and Huskins (1930) suggested that such linkage between chromosomes could be accounted for by sectional translocations along the lines of the theory developed by Darlington (1929) for Oenothera. If the chromosomes do have this unusual tendency to break-up and re-unite in the novel way suggested by Haldane the detection of linkage is made even more difficult.

Circumstantial evidence is presented in this thesis that the colour pattern polymorphism is determined by a series of linked loci rather than multiple alleles at the one locus:

(i) A number of individual locusts with colour patterns showing characteristic markings due to the presence of more than two "alleles" have been found (Page 83).

(ii) A calculation testing for multiple allelomorphism revealed differences from expected in all cases (Pages 84,85, Table 4.15) although the equation used was derived by assuming that loci were linked.

(iii) The argument developed on page 59 *et seq*, suggests the porphyrica phenotype may be determined at a separate locus.

(iv) The series of crosses resulting from an individual of the western race, page 47 *et seq*, tables 3.3 and 3.7 in particular, can only be interpreted in terms of at least two loci. However, it is necessary to make so many assumptions and qualifications in order to interpret the results within a Mendelian framework that the data become virtually worthless as supportive evidence for a model which postulates linked loci.

In summary, the evidence presented does not allow distinction between the hypotheses. In the interests of simplicity and taking into consideration the large number of crosses which segregated according to prediction (on a multiple allelic hypothesis) one would have to lean toward a series of multiple alleles. Nevertheless, it is useful to assume that the polymorphism is determined by a series of linked loci and use this as a basis to speculate (in a spirit of conjecture) on a number of related issues.

Two or more non-allelic genes may behave as pseudoalleles in inheritance <u>provided no recombination between them can be detected.</u> If the genes concerned are not closely linked physically on the chromosome it would still be possible for them to simulate close linkage, if chiasmata (resulting in crossing-over and recombination) are not formed between them. In fact, evidence presented in Chapter Five has revealed the presence of a very low chiasma frequency (5.4) coupled with a high degree of

chiasma localisation (5.5). Unfortunately, there are not enough markers to enable identification of the particular autosome (let along the precise location) carrying the genes determining the polymorphism. It would not be unexpected if the genes determining the colour pattern polymorphism in *Chortoicetes* were in a region of an autosome that was subject to infrequent recombination. Thus the relatively low number of chiasmata per cell allied with the high degree of localisation may act to inhibit detection of recombinant progeny. Moreover, where crossing-over is detected in a particular family it would not be surprising to detect recombinants among the progeny of sib-matings (as apparent) since the pattern and frequency of chiasma formation is also likely to be under genetic control (Barker, 1960; Rees, 1961). If it were possible to raise the chiasma frequency and vary the pattern of chiasma localisation, detection of recombinants may be made easier. In this context, cases are known where chiasma formation and frequency can be altered experimentally (Chandley, 1968; Henderson, 1969). It would be useful to employ these methods in conjunction with a further study of the inheritance of colour patterns particularly in the western race which seems to present the most hope for a successful solution.

In some of the other diverse organisms exhibiting polymorphism in which rare genes are dominant to their more common alleles (Page 131) a similar situation is found. For example, in *Paratettix texanus* where 23 of the genes

determining the colour patterns are closely linked on one pair of chromosomes, crossing-over takes place (although to a different extent) in both sexes (Nabours *et al*, 1933). On the other hand, in *Apotettix eurycephalus* all 15 dominant patterns and the universal recessive are inexplicably located along a very short section of the smallest pair of autosomes and crossing-over is exclusively restricted to the female (Nabours and Stebbins, 1950). A localised pattern of chiasma distribution may also be a common feature of the grouse locusts and no crossing-over has been detected in the male of *Apottetix* despite Nabours'(1914 *et seq*) extensive breeding experiments. It is probable that the apparent absence of crossing-over in the male of *Apottetix* as well as the difference in cross-over values between the two sexes of *Paratettix* results from a different type of chiasma localisation and/or a different overall chiasma frequency in the male and female. *Lebistes* also conforms to the general pattern. The majority of genes determining the polymorphic colour patterns normally behave as though linked to the Y chromosome - the mode of sex determination being of the XY male, XX female type. Two-hundred and thirty-three of the colour pattern genes have been found to be exclusively Y linked, 29 exclusively X linked and 20 to undergo crossing-over between the X and Y chromosomes (Haskins *et al*, 1961). Similarly, in *Cepaea nemoralis* there is a low chiasma frequency and localisation with respect to the A group bivalent has been found (Bantock, 1972). Cook (1967) has calculated that the probability of six of the nine loci involved in the colour and banding polymorphism of *Cepaea* lying on this chromosome is 4×10^{-7}. Although there is, at present, no

indication that this is indeed the case, it is clear that the chromosomes of *Cepaea*, and that either arm (but not both arms together) of the A chromosomes in particular, present an admirable vehicle for the evolution of large permanent linkage groups since the conditions required for a super-gene are satisfied (Bantock, 1972). The low frequency of cytological cross-overs in certain parts of the A group bivalent is not inconsistent with the low frequency of recombinants detected by breeding programmes. The alleles governing the general shell-colour, from brown to light yellow, and the pair determining whether or not shells are banded constitute a super-gene which must occupy only a very small part of the total available chromosomal space; if it is on either arm of the A chromosome then it presumably contains or is linked to numerous other loci not yet identified. Digressing for a moment, it is interesting to note that heterozygous advantage may arise not only through dominance and recessiveness, but also through the accumulation of disadvantageous recessives close to the switch-locus (Ford, 1975). Indeed, linkage between the colour and banding components appeared absolute until Cain *et al's* (1960) report in which one recombinant was detected among 46 progeny from two sibling snails (cross-over value 2.25 per cent). No follow-up data on this individual has ever been reported and it is presumed that the individual died before reaching maturity or left no progeny. Bantock (1972) also reported that a preliminary investigation of several other polymorphic populations of *Cepaea nemoralis*, and of the closely related *Cepaea hortensis*

suggested that the restriction to a single proximal chiasma in the A group bivalent is widespread in both species. Thus the inherent difficulties in detecting recombinant progeny among these species may result from the intrinsic nature and properties of the polymorphisms *per se*.

The polymorphisms which have been intensively studied may also be characteristic of whole Genera or families of animals. Thus Nabours (l.c.) noted a tendency for several of the same general patterns to be repeated in different genera and species. Similarly, the extent of the parallelism between the colour pattern polymorphism of *Chortoicetes* and some of its closely related *Austroicetes* spp. suggests that the basis for the polymorphism antedates speciation. In the gastropod molluscs such as *Cepaea*, palaeontological evidence indicates that the polymorphism for banding and shell colour has existed since at least the Pleiostocene era (Diver, 1929). Moreover, it occurs in both terrestrial (*Cepaea*, *Hemitrochus*, *Liguus*) and marine genera (*Littorina*, *Thais*) (Mayr, 1963). The wide distribution of similar polymorphisms throughout entire families and orders, suggests that these polymorphisms not only have important selective value, but that they are of considerable phylogenetic antiquity. It is probable that at some stage in the evolutionary past, some heterotic mechanism became associated with phenotypic polymorphism and was subsequently maintained in the entire group.

It may be that the colour pattern polymorphisms have some concealing affect affording protection from predators; the disruptive patterns being composed of different colours and/or contrasting shades of the same colour. For this cryptic colouration to be effective, each organism would be expected to behave according to its colour pattern- i.e. individuals must rest on a suitable background and often orientate themselves in a particular way relative to their surroundings to gain a camouflage effect. Thus some protectively coloured species such as melanic and non-melanic forms of some moths tend to rest on an appropriately coloured background while in other species such as *Cepaea nemoralis* there is no evidence for this (Ford, 1975). Rubtzov (1935) recorded that the rubiginosa colour pattern in *Chorthippus albomarginatus* was relatively more frequent in dry than in humid habitats where it is afforded protection from predators by virtue of a camouflage effect. Descriptions of this colour pattern suggest that its appearance is very similar to the rubiginosa pattern of *Chortoicetes*. The pleiotropic nature of the genes determining the polymorphism may provide the answer. Survival of a gene in a gene pool depends on its total contribution to the fitness, not just on the contribution to fitness made by the visible phenotype. Thus, the colour patterns may be merely an incidental by-product of genes maintained in the gene pool for other physiological properties.

Consideration of the inheritance of the porphyrica pattern in *Chortoicetes* gives added insight into the mechanism controlling the colour-pattern polymorphism. Although this

colour pattern rarely constitutes more than a few per cent, Ford (1945) would nevertheless consider that it possessed some advantage.

The rarity of the porphyrica gene combined with selection against the phenotype suggests intuitively that this gene should be eliminated from each population in which it is found. If the pattern is determined by a separate locus then its continued existence is explicable in terms of Ewens' (1969) analysis of linkage. In short, Ewens has shown that provided 'linkage is sufficiently close, a less-fit gene may steadily replace a more-fit one, when the former is of rare occurrence'.

Although it has been shown that dominance can be selected for (Ford, 1940; Fisher and Holt, 1944), this does not prove that it is normally evolved and that disadvantageous mutants usually become recessive as suggested by Fisher (1928, *et seq*). That they usually do so may be indicated by the fact that the common allelomorph found in natural populations is nearly always dominant for its major effects. In theory, it is possible to test Fisher's (l.c.) hypothesis on the evolution of dominance by putting dominant mutants from one species into the gene-complex of another in which they have not occurred, to see if dominance vanishes. There are, however, several difficulties with this approach. To begin with the test requires successful inter-species hybridisation. It is also impossible to be certain that the mutant does not occur, or has not in the past, in the test stock. When two forms can be crossed to produce fertile hybrids they are likely to have arisen from a common ancestral stock in the not too distant past, and the mutant may well have occurred in this stock.

Nevertheless, several such tests have been achieved. Ford (1955) reported a breakdown in dominance in racial crosses of a dimorphic form in the moth *Triphaena comes*. Similarly, Kettlewell (1965) found a breakdown of dominance in a complete dominant melanic form of *Biston betularia* after three generations of out-crossing to the closely related North American species *Biston cognataria*. In other crosses involving a different gene-complex the breakdown was avoided. Cadbury (1975) provides an example of a gene completely recessive in effect in *Lycia hirtaria* which when crossed to a species of a related genus (*Nyssia zonaria*) was clearly recognisable in the F_1 progeny. Thus dominance and recessiveness are the product of selection acting on the gene-complex, not of the genes responsible for the characters which display these qualities as suggested by Wright (1934) and Crosby (1963). Although the continued backcrossing of the dominant porphyrica eastern form into the western race did not result in a breakdown of dominance it is not conclusive evidence that the character may be dominant irrespective of its genetic background. As reported above for *Biston betularia* in a different gene complex the breakdown may occur. It would be interesting to ascertain dominance relationships by out-crossing *Chortoicetes* to the various related *Austroicetes* species.

Although a case of variable dominance in a laboratory discovered mutant of *Drosophila melanogaster* has previously been reported by Whitten (1968), variable dominance in the porphyrica phenotype between the two races of *Chortoicetes* constitutes the first ever reported case of a character determined by variable dominance in the wild. In testing

the mode of inheritance of this pattern in the laboratory an important dilemma rose. If the patterns are determined by a series of alleles at the one locus and porphyrica is also allelic, then the western race porphyrica allele must be dominant over the eastern race nigrovirgata form (Page 59) and may also be dominant with respect to trilineata and albomedia (the latter two combinations having not been tested). In the western race, porphyrica is recessive to all other alleles, including rubiginosa. On the other hand, the eastern race porphyrica type showed exactly the same dominance relationship in both races. Considering the alternative hypothesis of porphyrica being determined by a separate locus, the western race porphyrica form would be dominant over the wild type eastern allele, nigrovirgata being epistatic and not expressed in the presence of the porphyrica gene. Despite intensive back-crossing of eastern porphyrica forms no recombinant progeny were detected and it is thus not possible to distinguish between the two hypotheses. The situation may become clearer if a further study of porphyrica individuals from the western race with the dominant patterns of the eastern race proves possible in the future.

Haldane (1930) suggested that the correlation between the dominant types and excessive linkage (features of the species mentioned in this chapter) is explained by the fact that the dominant types differ from normal 'not by single genes, but by duplication or translocation of whole sections of chromosomes'. In effect he postulates that the dominant

genotypes are due to the duplication of such translocated segments. The form resulting from the duplicated segment would be dominant on Haldane's hypothesis because, as the locus would be represented two or more times in the chromosomes, it would produce more enzyme than the equivalent unduplicated segment. The suggestion is 'extremely attractive' in terms of the peculiar linkage observed (as discussed earlier) but it is not clear in what manner Haldane finds it an explanation of dominance (Fisher, 1930b). According to this theory,some of the genes in the duplication would be expected to 'have a greater effect when three or four are present than when only two are found, as in the normal type' (Haldane, l.c.). This explains why the heterozygous duplication differs from the universal recessive, but it does not explain the complete dominance exhibited by the albomedia gene, i.e. why the homozygous duplication A/A should resemble the heterozygote A/R. This fact is even more marked in the grouse locusts. To explain this it is necessary to assume that 'some of the genes in the duplication have a greater effect when three are present, but that none of them should exert a further effect when the three are increased to four; and this curious type of limitation must be observed in each of the possible duplications to which the different dominants are to be ascribed' (Fisher, 1930b). Although Haldane's (l.c.) hypothesis explains the linkage phenomenon, it does not appear to be a valid explanation of the dominance exhibited by these groups.

If Haldane's suggestion of duplication is accepted then 'on Fisher's theory there is no obvious reason why modifers should not be able to suppress the effect of a

duplication as easily as that of a gene'. (Haldane, l.c.). Fisher (1930b) cites two reasons why dominance modification by selection should be particularly effective in these cases. Firstly, such polymorphism results in the production of large numbers of heterozygotes increasing the efficacy of selection and overcoming the objections of Wright (1929a,b; 1934a,b) and Haldane (1930) to the magnitude of selective intensity. Secondly, if the dominant forms are due to a duplication, the evolutionary modification of the relevant tract of chromatin will take place almost wholly in heterozygous individuals .

Ignoring duplication as a cause of the dominant characters then a similar result will follow if, as is made probable by the observed linkage phenomena, each gene is situated in a chromosome section where crossing-over occurs rarely or not at all. The change in such a section due to evolution would then be dependent on its effects in the presence of a 'dominant gene'. Modification of dominance through evolutionary change in 'permanently' associated genes must therefore resemble that of multiple alleles in a similar way to that suggested by Haldane (1939). In this context it is interesting to note that the retention of dominance by the eastern race porphyrica gene when successively "backcrossed" to western race individuals accords with Haldane's (1930, 1939) hypothesis, i.e. that dominance is evolved by selection of the genotype defending the organism against external environmental influences and genotype variation.

CONCLUSION

Examination of the colour pattern polymorphism of the Australian Plague Locust has resulted in a number of findings which require further investigation. The polymorphism seems to be maintained by the selective advantage of heterozygous over homozygous forms, the gene frequencies in stable equilibrium being determined by the balance of selective forces. Consequently, the frequencies of the various polymorphic forms have been found to differ with geographical and seasonal variation.

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CHAPTER TWO

APPENDICES

Appendix 2.1

Egg pods collected from the Canberra population which is found at Point Hut.

Egg Pod	Number of Eggs*
C 1	38
C 2	33
C 3	31
C 4	33
C 5	27
C 6	29
C 7	34
C 8	20
C 9	32
C 10	18
C 11	29
C 12	20
C 13	23
C 14	21
C 15	22
C 16	29
C 17	35
C 18	28
C 19	28
C 20	43
C 21	41
C 22	37
C 23	44
C 24	39
Total eggs	737

* All eggs collected were of the diapause variety.

CHAPTER THREE

APPENDICES

COLOUR PATTERN GENOTYPE FREQUENCIES AMONG OFFSPRING FROM INDIVIDUAL MATINGS CATEGORISED BY SEX AND COLOUR PATTERN

1. EASTERN RACE

$\frac{A}{A}$ ♂ X $\frac{A}{A}$ ♀

Mating Number	A/A Male	Female	Total	X_1^2
0	5	3	8	0.1250
1	7	5	12	0.0832
2	7	8	15	0.0000
3	9	10	19	0.0000
4	4	1	5	0.8000
5	4	6	10	0.1000
6	9	8	17	0.0000
7	7	5	12	0.0832
8	8	6	14	0.0714
9	4	3	7	0.0000
10	7	7	14	0.0714
11	8	7	15	0.0000
12	16	18	34	0.0294
13	12	13	25	0.0000
14	7	4	11	0.3636
15	12	9	21	0.1904
16	9	6	15	0.2666
17	10	12	22	0.0454
18	11	12	23	0.0000
19	10	10	20	0.0500
20	13	11	24	0.0416
21	14	13	27	0.0000
22	9	9	18	0.0554
23	3	4	7	0.0000
24	5	8	13	0.3076
25	13	13	26	0.0384
26	5	2	7	0.5714
27	20	5	25	7.8400 **
28	7	9	16	0.0624
29	10	8	18	0.0554
30	4	7	11	0.3636
31	7	5	12	0.0832
32	3	5	8	0.1250
33	6	2	8	1.1250
34	4	2	6	0.1666
35	6	9	15	0.2666
36	12	16	28	0.3214
37	11	13	24	0.0416
38	13	9	22	0.4091
TOTAL	331	303	634	1.1498

$\frac{N}{N}$ ♂ X $\frac{N}{N}$ ♀

Mating Number	N/N Male	Female	Total	X_1^2
200	11	14	25	0.1600
201	10	10	20	0.0500
202	4	1	5	0.8000
203	1	5	6	1.5000
204	6	7	13	0.0000
205	3	4	7	0.0000
206	7	5	12	0.0832
207	5	7	12	0.0832
208	7	9	16	0.0624
209	8	8	16	0.0624
210	5	8	13	0.3076
211	1	3	4	0.2500
212	6	14	20	2.4500
213	7	10	17	0.2352
214	7	9	16	0.0624
215	8	12	20	0.4500
216	16	16	32	0.0312
217	18	13	31	0.5160
218	8	9	17	0.0000
219	15	9	24	1.0416
220	10	13	23	0.1738
221	8	12	20	0.4500
222	10	13	23	0.1738
223	17	12	29	0.5516
224	16	11	27	0.5924
225	11	14	25	0.1600
226	10	8	18	0.0554
227	5	4	9	0.0000
228	12	15	27	0.1480
229	14	13	27	0.0000
230	3	1	4	0.2500
231	6	7	13	0.0000
TOTAL	275	296	571	0.7004

$\frac{R}{R}$ ♂ X $\frac{R}{R}$ ♀

Mating Number	R/R Male	Female	Total	X_1^2
350	5	4	9	0.0000
351	18	18	36	0.0000
352	13	14	27	0.0000
353	12	8	20	0.4500
354	9	18	27	2.3702
355	7	14	21	1.7142
356	9	12	21	0.1904
357	9	11	20	0.0500
358	1	9	10	4.9000*
359	4	4	8	0.1250
360	6	4	10	0.1000
361	9	8	17	0.0000
362	7	5	12	0.0832
363	10	10	20	0.0000
364	9	8	17	4.7618*
365	5	16	21	0.0454
366	10	12	22	0.5000
367	7	11	18	0.0000
368	13	12	25	1.3888
369	6	12	18	0.0356
370	15	13	28	1.5000
371	5	1	6	0.0000
372	6	5	11	0.2250
373	22	18	40	0.4102
374	17	22	39	0.0454
375	12	10	22	0.0294
376	18	16	34	0.0356
377	15	13	28	0.0000
378	16	17	33	0.0332
379	16	14	30	0.2500
380	20	16	36	0.2500
381	20	16	36	3.2258
382	21	10	31	0.0312
383	16	16	32	0.6400
384	15	10	25	0.0000
385	11	10	21	0.1212
386	18	15	33	0.0000
387	10	9	19	0.2500
388	16	20	36	2.2068
389	19	10	29	0.3750
390	14	10	24	0.7618
391	13	8	21	0.0714
392	8	6	14	3.7812
393	22	10	32	0.0238
394	22	20	42	0.1904
395	12	9	21	0.3214
396	16	12	28	0.0356
397	14	14	40	2.0250
398	25	15	14	0.0714
399	8	6		
TOTAL	631	581	1212	1.9810

$\frac{T}{T}$ ♂ X $\frac{T}{T}$ ♀

Mating Number	T/T Male	Female	Total	X_1^2
450	4	4	8	0.1250
451	7	4	11	0.3636
452	6	4	10	0.1000
453	8	10	18	0.0554
454	6	6	12	0.0832
455	3	4	7	0.0000
456	5	1	6	1.5000
457	18	14	32	0.2812
458	8	10	18	0.0554
459	12	10	22	0.0454
460	4	5	9	0.0000
461	11	2	13	4.9230*
462	3	1	4	0.2500
463	7	3	10	0.9000
464	5	15	20	4.0500
465	8	6	14	0.0714
466	8	10	18	0.0554
467	5	5	10	0.1000
468	9	8	17	0.0000
469	9	18	27	2.3702
470	17	11	28	0.8928
471	6	6	12	0.0832
472	8	13	21	0.7618
473	10	15	25	0.6400
474	7	12	19	0.8420
475	4	12	16	3.0624
476	4	3	7	0.0000
477	12	13	25	0.0000
478	10	11	21	0.0000
479	11	10	21	0.0000
480	24	8	32	8.0000 **
481	14	19	33	0.4846
482	6	3	9	0.4444
483	10	7	17	0.2352
484	13	4	17	3.7646
485	19	9	28	2.8928
486	10	11	21	0.0000
487	10	16	26	0.9614
488	9	7	16	0.0624
489	3	2	5	0.0000
490	5	7	12	0.0852
491	1	2	3	0.0000
492	6	4	10	0.1000
493	7	10	17	0.2352
494	10	7	17	0.2352
495	4	2	6	0.1666
TOTAL	386	364	750	0.5880

$$\frac{A}{N}\ \sigma^{\prime} \times \frac{A}{N}\ \varphi$$

Mating Number	A/A		A/N		N/N		Total	X_5^2
	Male	Female	Male	Female	Male	Female		
50	2	0	4	4	2	3	15	0.7247
51	4	3	6	5	3	3	24	0.4999
52	0	1	1	0	2	3	7	1.8034
53	3	2	6	5	4	3	23	0.8258
54	2	2	5	5	1	2	17	0.8820
55	2	1	4	3	1	1	12	0.9997
56	3	2	8	7	4	3	27	0.9996
57	1	4	4	4	2	3	18	2.4442
58	1	2	8	9	4	2	26	3.9996
59	1	1	3	2	6	2	15	10.8664
60	5	4	8	4	6	5	32	3.5000
61	7	6	8	9	3	3	36	2.9999
62	2	1	4	6	2	2	17	1.3526
63	4	3	7	9	5	10	38	7.2629
64	3	1	7	4	0	1	16	5.7500
65	2	3	5	2	0	1	13	4.5381
66	2	2	6	11	4	3	28	3.8569
67	2	4	5	8	2	7	28	5.5712
68	0	1	5	8	1	2	17	6.7632
69	4	4	7	9	3	4	31	0.4836
TOTAL	50	47	111	114	55	63	440	2.9361

$\frac{A}{R}$ ♂ X $\frac{A}{R}$ ♀

Mating Number	R/R		A/A:A/R		Total	X^2_3
	Male	Female	Male	Female		
100	2	4	2	2	10	8.1332*
101	7	9	25	15	56	3.0475
102	1	2	1	1	5	4.0664
103	0	4	0	11	5	21.1330***
104	1	1	5	1	8	2.6666
105	0	0	4	5	9	3.1479
106	4	2	9	6	21	1.4761
107	3	4	13	19	39	2.3673
108	0	0	1	3	4	2.6666
109	5	2	0	7	14	11.9047**
110	4	2	8	7	21	0.9681
111	0	1	3	4	8	1.3333
112	2	2	4	9	17	19.4111***
113	4	1	5	6	16	2.6666
114	1	1	8	11	21	3.2537
115	1	2	1	3	7	2.5236
116	1	4	14	10	29	2.9078
117	4	3	6	7	20	1.3333
118	2	3	4	2	11	3.3027
119	5	3	9	6	23	2.3912
120	5	5	11	14	35	0.5807
121	3	4	6	7	20	1.3333
122	5	4	7	3	19	6.4033
123	0	2	5	3	10	2.2666
124	2	4	6	10	22	1.7574
125	1	1	7	3	12	2.2220
126	10	3	6	6	25	17.5596***
127	1	4	6	8	19	2.1928
128	6	2	4	3	15	10.7775*
129	2	3	12	14	31	1.6019
130	6	5	13	8	32	2.6666
131	1	0	3	1	5	1.9330
132	4	9	10	11	34	6.1567
133	2	2	6	7	17	0.0978
134	0	1	7	5	13	2.7946
135	1	1	11	5	18	4.5184
136	2	7	7	12	28	5.5236
137	1	2	5	9	17	1.9801
138	3	4	13	9	29	0.8848
139	2	3	6	8	19	0.5086
TOTAL	104	116	273	271	764	6.6316

$$\frac{A}{T}\ \male \times \frac{A}{T}\ \female$$

Mating Number	A/A		A/T		T/T			
	Male	Female	Male	Female	Male	Female	Total	X^2_5
150	0	3	2	5	1	1	12	3.500
151	1	1	4	1	0	0	7	3.2499
152	3	1	2	3	1	0	10	2.7500
153	1	1	1	1	1	1	6	0.6666
154	1	3	3	7	1	1	16	4.5000
155	1	2	3	1	2	4	13	5.3168
156	6	5	13	16	9	6	55	6.2124
TOTAL	13	16	28	34	15	13	119	1.2687

$\frac{N}{R}$ ♂ x $\frac{N}{R}$ ♀

Mating Number	N/N		N/R		R/R		Total	X_5^2
	Male	Female	Male	Female	Male	Female		
250	3	2	4	7	4	4	24	1.8331
251	2	1	5	5	2	2	17	0.8820
252	1	1	3	5	1	2	13	1.7689
253	2	2	5	4	2	3	18	0.4441
254	4	4	5	5	2	3	23	1.3476
255	0	0	4	2	1	0	7	2.9463
256	2	2	2	3	2	2	13	0.8459
257	4	4	5	5	2	3	23	1.3476
258	0	0	4	3	1	0	7	2.9463
259	0	2	5	4	1	2	14	1.1070
260	2	1	5	5	0	1	14	1.9641
261	1	1	4	6	2	1	15	2.5998
262	1	2	10	8	3	4	28	3.9998
263	1	2	6	1	1	1	12	4.9997
264	2	0	3	8	1	1	15	7.6664
265	1	2	2	3	1	3	12	2.3331
266	1	2	3	5	2	1	14	1.4284
267	1	2	7	4	1	2	17	2.9997
268	4	2	6	8	1	3	24	2.6665
269	2	2	8	6	2	3	23	1.6952
270	0	1	2	3	2	1	9	2.1107
271	1	2	2	5	1	0	11	3.9087
272	1	4	3	10	1	5	24	8.4998
273	0	0	1	2	2	1	6	3.9998
274	4	3	6	10	2	0	25	6.0396
275	2	4	12	12	2	2	34	6.4702
276	3	2	9	9	2	2	27	3.2219
277	1	3	10	12	2	2	30	7.3331
TOTAL	46	53	141	159	46	54	499	22.6510**

$\frac{N}{T}$ ♂ X $\frac{N}{T}$ ♀

Mating Number	N/N Male	N/N Female	N/T Male	N/T Female	T/T Male	T/T Female	Total	X^2_5
300	1	2	0	1	1	1	6	3.9998
301	4	6	3	9	2	4	28	5.4284
302	6	7	6	11	3	4	37	3.7563
303	2	4	7	4	3	0	20	4.6000
304	6	5	8	10	4	6	39	0.9999
305	1	3	1	7	1	4	17	7.4702
306	1	4	7	8	6	5	31	3.7094
307	3	3	5	7	0	4	22	3.8179
308	0	2	1	2	0	1	6	3.9998
309	1	2	2	3	1	2	11	0.9996
310	9	2	18	6	7	3	45	12.4220**
311	3	5	12	6	5	4	35	2.7139
312	4	4	11	3	3	2	27	5.5923
313	1	3	2	7	0	0	13	9.4611
314	5	5	8	8	5	4	35	0.3973
315	4	3	10	10	0	0	27	10.0367
316	3	3	8	10	2	2	28	2.8569
317	6	4	9	7	1	2	29	4.6549
318	7	3	11	8	6	2	37	4.1888
319	6	3	9	4	1	2	25	6.5196
320	2	4	6	3	2	5	22	3.9997
321	3	4	10	4	2	2	25	4.1196
322	5	2	8	6	6	4	31	2.8062
323	5	2	1	2	2	1	13	9.4612
324	2	3	4	9	1	2	21	4.3331
325	2	1	8	4	4	4	23	3.7824
326	2	0	14	11	6	10	43	12.5345*
327	4	1	9	4	4	2	24	4.4998
328	5	3	8	9	7	2	34	3.5292
329	0	1	7	3	2	3	16	5.5000
330	2	0	5	6	2	2	17	2.9996
331	3	2	8	7	3	3	26	0.9228
332	5	4	6	8	4	5	32	1.0000
333	1	4	8	9	7	4	33	4.4541
334	5	1	9	7	4	3	29	4.4479
335	0	2	4	4	5	1	16	7.0000
336	1	1	11	11	0	0	24	16.9998**
337	2	3	4	7	2	4	22	1.8178
338	3	4	6	1	2	1	17	5.8231
339	1	2	9	5	5	4	26	4.4612
340	2	4	4	8	0	1	19	6.4461
341	0	0	6	8	2	3	19	7.2541
342	1	2	8	6	3	1	21	3.7616
TOTAL	129	123	301	273	126	119	1071	7.4087

Appendix 3.10

$\frac{R}{T}$ ♂ x $\frac{R}{T}$ ♀

Mating Number	R/R		R/T		T/T		Total	X_5^2
	Male	Female	Male	Female	Male	Female		
400	6	4	12	2	2	0	26	10.7497
401	1	2	5	7	3	5	23	3.4345
402	1	3	6	6	2	1	19	2.4734
403	1	1	8	7	1	1	19	6.4733
404	6	3	7	9	2	2	29	3.5515
405	1	2	5	6	1	0	15	2.1998
406	1	2	3	6	2	2	16	1.7500
407	3	5	10	4	3	2	27	4.2589
408	4	3	9	8	3	3	30	0.7999
409	4	5	4	6	2	1	22	4.1815
410	2	4	8	6	3	2	25	1.5596
411	0	2	1	5	0	1	9	4.0943
412	4	2	8	6	2	1	23	3.0866
413	3	2	3	4	4	2	18	2.2220
414	3	4	8	6	2	3	26	1.0766
415	3	1	2	8	2	1	17	6.0584
416	4	3	8	10	3	5	33	1.1792
417	0	1	2	8	4	4	19	1.5591
418	1	0	4	3	0	0	8	2.5000
419	3	3	3	3	1	2	15	2.0644
420	4	4	3	3	1	1	16	5.5000
421	2	1	3	2	1	1	10	0.8000
422	3	5	12	10	7	5	42	1.8093
423	0	1	4	4	4	4	17	9.9324
424	1	0	4	3	2	1	11	1.0792
425	4	1	4	8	5	2	24	4.6664
426	2	5	6	7	0	0	20	3.6000
427	4	3	8	7	3	2	27	0.9996
428	4	3	8	5	1	3	24	2.4998
429	5	1	7	7	1	2	23	4.8258
430	1	1	5	3	2	2	14	1.4284
431	4	2	4	6	5	8	29	8.2410
432	1	2	4	10	2	4	23	5.9617
433	3	4	10	6	4	4	31	1.2578
434	2	2	4	4	2	4	18	1.5552
435	2	3	6	5	2	5	23	2.2170
436	4	3	2	6	0	1	16	7.0000
437	1	1	5	6	1	0	14	5.1427
TOTAL	98	94	215	222	85	87	801	7.8736

COLOUR PATTERN GENOTYPE FREQUENCIES

AMONG OFFSPRING FROM INDIVIDUAL

MATINGS CATEGORISED BY SEX AND

COLOUR PATTERN

2. WESTERN RACE

$\frac{R}{R}$ ♂ x $\frac{R}{R}$ ♀

Mating Number	R/R Male	Female	Total	χ^2_1
2001	4	7	11	0.3636
2002	9	11	20	0.0500
2003	14	12	26	0.0384
2004	4	10	14	1.7856
2005	11	12	23	0.0000
2006	6	3	9	0.4444
2007	15	22	37	0.9728
2008	9	8	17	0.0000
2009	10	7	17	0.2352
2010	6	1	7	2.2856
2011	16	9	25	1.4400
2012	4	2	6	0.1666
2013	13	12	25	0.0000
2014	11	14	25	0.1600
2015	14	9	23	0.6956
TOTAL	146	139	285	0.1262

$\frac{N}{N}$ ♂ x $\frac{N}{N}$ ♀

N/N

Mating Number	Male	Female	Total	X_1^2
2050	16	14	30	0.0332
2051	5	5	10	0.1000
2052	12	7	19	0.8410
2053	8	13	21	0.7618
2054	12	15	27	0.1480
2055	12	12	24	0.0416
2056	10	4	14	1.7856
2057	15	10	25	0.6400
2058	10	9	19	0.0000
2059	12	15	27	0.1480
TOTAL	112	104	216	0.2268

$$\frac{T}{T} \; ♂ \quad X \quad \frac{T}{T} \; ♀$$

Mating Number	T/T Male	Female	Total	X_1^2
2100	1	5	6	1.5000
2101	14	7	21	1.7142
2102	1	4	5	0.8000
2103	7	1	8	3.1250
2104	7	6	13	0.0000
2105	18	10	28	1.7500
2106	14	5	19	3.3684
2107	7	10	17	0.2352
TOTAL	69	48	117	3.4188

$\frac{A}{N}$ ♂ x $\frac{A}{N}$ ♀

Mating Number	A/A		A/N		N/N			
	Male	Female	Male	Female	Male	Female	Total	X_5^2
2150	5	0	8	7	4	3	27	4.5552
2151	2	2	5	5	1	3	18	1.1108
2152	1	2	2	4	0	0	9	4.3329
2153	0	1	2	2	2	2	9	2.5551
2154	3	2	6	4	3	3	21	0.7140
2155	1	3	2	5	2	3	16	2.7500
2156	6	3	2	8	1	3	23	7.9563
2157	3	0	5	4	7	2	21	10.4283
2158	0	1	0	3	0	1	5	5.3996
TOTAL	21	14	32	42	20	20	149	2.9998

$$\frac{A}{R}\ \sigma^{\prime} \times \frac{A}{R}\ \varphi$$

Mating Number	R/R		A/A;A/R			
	Male	Female	Male	Female	Total	X^2_3
2200	1	2	12	11	26	2.7177
2201	0	1	3	5	9	1.9627
2202	1	0	2	4	7	1.7617
2203	2	1	2	0	5	5.8830
2204	0	0	1	3	4	2.6666
2205	2	0	9	10	21	3.5078
2206	1	1	4	4	10	0.1332
2207	3	3	2	10	18	5.4073
2208	1	3	6	9	19	1.6314
2209	2	3	3	8	16	2.6666
2210	1	3	8	11	23	1.9273
2211	1	4	5	5	15	2.9554
2212	7	2	8	4	21	9.3490*
TOTAL	22	23	65	84	194	2.8383

$\frac{N}{R}$ ♂ x $\frac{N}{R}$ ♀

Mating Number	N/N		N/R		R/R			X_5^2
	Male	Female	Male	Female	Male	Female	Total	
2250	4	2	6	8	1	3	24	2.6665
2251	3	2	3	6	2	3	19	2.6138
2252	4	5	9	9	3	6	36	1.1110
TOTAL	11	9	18	23	6	12	79	2.8731

$\frac{R}{T}$ ♂ X $\frac{R}{T}$ ♀

Mating Number	R/R		R/T		T/T		Total	X^2_5
	Male	Female	Male	Female	Male	Female		
2300	6	3	19	7	4	3	42	10.3806
2301	3	1	4	7	5	6	26	5.8459
2302	0	0	4	1	3	0	8	9.5000
2303	3	2	9	11	4	3	32	2.7500
2304	5	2	10	8	6	4	35	2.2567
2305	1	2	0	1	6	3	13	18.0766**
2306	0	1	0	3	2	0	6	6.6666
2307	1	4	2	6	1	1	15	5.7998
2308	3	3	9	7	4	2	28	1.4284
2309	3	3	10	7	4	2	29	2.0342
2310	0	0	1	1	0	1	3	2.3329
2311	1	1	2	1	2	4	11	6.8178
2312	4	3	7	10	3	3	30	1.3332
2313	2	1	4	4	2	0	13	2.3842
2314	3	4	5	11	4	3	30	2.7998
2315	2	2	4	2	3	3	16	2.0000
2316	0	1	1	1	2	1	6	3.3331
2317	5	3	7	5	1	3	24	2.9998
2318	4	1	8	7	1	2	23	4.3041
2319	2	4	3	11	2	1	23	8.3040
2320	3	0	3	2	2	3	13	4.5381
2321	0	0	2	3	1	0	6	3.9999
2322	3	4	6	9	4	4	30	0.7998
2323	2	1	4	8	4	12	31	21.9029***
2324	4	4	7	7	5	4	31	0.4836
2325	6	1	5	6	2	4	24	5.1665
2326	4	5	9	4	5	6	33	3.4845
2327	4	3	14	15	5	5	46	3.6520
2328	4	2	5	4	3	4	22	1.8178
2329	1	2	0	0	2	1	6	7.3332
2330	0	2	8	5	2	3	20	4.6000
2331	2	1	4	5	3	2	17	1.1173
2332	0	1	4	3	2	3	13	3.3073
2333	1	1	10	10	2	2	26	7.8458
2334	2	1	6	4	3	1	17	2.2937
2335	4	2	5	5	4	2	22	1.6360
2336	1	0	6	4	5	1	17	7.9407
2337	2	1	5	8	3	4	23	2.9128
2338	4	4	4	8	3	4	27	1.7404
2339	3	3	5	2	5	1	19	5.6313
2340	2	5	1	0	3	6	17	18.0584**
2341	1	3	3	10	2	5	24	7.1665
2342	1	1	7	5	3	4	21	3.3807
TOTAL	102	88	232	238	132	126	918	11.6904*

COLOUR PATTERN GENOTYPE FREQUENCIES AMONG OFFSPRING FROM INDIVIDUAL MATINGS CATEGORISED BY SEX AND COLOUR PATTERN.

3. PORPHYRICA MATINGS - EASTERN RACE

$\frac{R}{P}$ ♂ x $\frac{R}{P}$ ♀

($\frac{R}{R}$)

Mating Number	Non-Porphyrica Male	Non-Porphyrica Female	Porphyrica Male	Porphyrica Female	Total	X_3^2
600	10	6	22	25	63	1.2124
601	6	6	21	16	49	0.6868
602	10	5	11	19	45	5.7849
603	1	4	5	9	19	3.8723
604	2	5	31	28	66	8.2000
605	6	6	16	18	46	0.3005
606	3	2	20	14	39	4.4187
607	11	2	24	12	49	10.5916*
608	2	3	10	11	26	0.6665
609	10	6	26	19	61	2.1693
610	3	1	11	12	27	2.1356
611	2	2	8	10	22	0.7877
612	1	1	5	1	8	2.6666
613	3	4	8	8	23	0.5360
164	15	6	20	19	60	8.6221*
615	1	1	11	13	26	4.3587
616	2	3	10	12	27	0,9504
617	3	2	11	3	19	4.7191
618	1	4	9	4	18	3.9258
619	2	1	5	4	12	0.4442
620	5	1	7	11	24	3.5554
621	2	1	11	11	25	2.4130
622	4	2	23	13	42	6.1269
623	1	1	5	1	8	2.6666
624	5	1	7	11	24	3.5554
625	2	1	11	11	25	2.4130
626	2	1	5	4	12	0.4442
627	4	2	23	13	42	6.1269
628	5	4	10	10	29	0.7010
629	3	1	8	7	19	1.0699
630	1	2	6	5	14	0.4761
631	4	5	9	11	29	0.8848
632	2	5	7	13	27	3.1233
633	4	5	9	12	30	0.9332
634	1	3	10	9	23	1.4637
635	7	4	16	14	41	1.0811
636	5	3	12	13	33	0,5352
637	3	1	11	8	23	1.9273
638	5	5	14	12	36	0.8333
639	3	1	8	7	19	1.0699
640	5	4	10	10	29	0.7010
641	1	1	5	5	12	0.4442
642	3	4	11	12	30	0.2221
643	3	4	8	12	27	0.9504
644	3	2	14	11	30	1.6443
645	2	1	9	5	17	1.9801
646	1	2	2	2	7	1.7618
647	5	5	7	3	20	7.7333
648	3	3	8	6	20	0.5333
649	4	3	16	12	35	1.1903
TOTAL	192	148	586	531	1457	10.2357 *

$\frac{N}{P}$ ♂ x $\frac{N}{P}$ ♀

($\frac{N}{N}$)

Appendix 3.20

Mating Number	Non-Porphyrica		Porphyrica			
	Male	Female	Male	Female	Total	χ^2_3
550	0	1	2	0	3	3.2219
551	4	3	9	10	26	0.2562
552	5	3	10	9	27	0.9504
553	3	1	11	11	26	1.8972
554	3	3	7	13	26	1.8973
555	5	3	5	4	17	5.4311
556	2	3	4	5	14	1.2380
557	2	1	1	1	5	4.0664
558	1	1	3	4	9	0.1849
559	2	1	4	5	12	0.4442
560	0	0	5	5	10	3.3332
561	1	1	8	11	21	3.2537
562	0	3	6	9	18	3.3333
563	1	1	8	3	13	3.2049
564	1	2	4	3	10	0.6666
565	0	0	8	1	9	10.2589*
566	1	1	6	12	20	4.8000
567	3	5	13	7	28	2.4760
568	4	3	6	10	23	1.4637
569	0	2	5	13	20	7.4666
TOTAL	38	38	125	136	337	1.5557

$\frac{R}{P}$ ♂ X $\frac{T}{P}$ ♀

($\frac{R}{T}$)

Mating Number	Non-Porphyrica Male	Non-Porphyrica Female	Porphyrica Male	Porphyrica Female	Total	X_3^2
750	3	3	5	6	17	1.0389
751	2	2	8	11	23	1.2315
752	3	3	9	13	28	0.9522
753	2	0	4	4	10	0.4832
754	2	2	6	1	11	3.7876
755	0	2	4	2	8	1.6666
756	3	3	9	13	28	0.9522
757	2	0	4	4	10	0.4832
758	1	2	8	11	22	2.2422
759	1	2	6	5	14	0.4761
760	5	5	6	10	26	3.3333
761	3	6	8	8	25	3.0530
762	3	4	15	12	34	0.8234
763	3	3	7	12	25	1.3463
764	5	7	12	14	38	1.4383
765	4	3	13	12	32	0.3333
766	2	1	8	6	17	1.0390
767	3	1	4	2	10	3.3332
768	2	4	11	12	29	0.8848
769	1	1	2	2	6	0.2220
770	3	2	6	5	16	0.6666
771	4	2	13	10	29	1.2527
772	2	0	3	2	7	1 6487
773	2	1	5	3	11	0.8786
774	1	2	6	7	16	0.6666
775	2	1	7	12	22	3.2119
776	0	3	5	5	13	1.1698
777	1	2	2	4	9	1.3701
778	1	0	7	3	11	2.4127
779	0	2	5	4	11	1.8482
780	2	3	2	4	11	3.3027
781	2	2	5	10	19	1.9121
782	2	0	3	8	13	4.4357
783	4	1	6	2	13	5.6665
784	4	3	8	5	20	1.8666
785	3	3	7	4	17	
TOTAL	83	81	239	248	651	0.2031

$\frac{A}{P}$ ♂ X $\frac{A}{P}$ ♀

Mating Number	Non-Porphyrica ($\frac{A}{A}$) Male	Non-Porphyrica ($\frac{A}{A}$) Female	Porphyrica ($\frac{A}{P}$) Male	Porphyrica ($\frac{A}{P}$) Female	Porphyrica ($\frac{P}{P}$) Male	Porphyrica ($\frac{P}{P}$) Female	Total	X_5^2
500	3	3	4	2	1	1	14	3.1426
501	1	2	2	3	2	2	12	0.9997
502	4	2	5	9	5	1	26	4.4612
503	7	4	11	12	0	5	39	1.7568
504	2	0	6	6	1	0	15	3.1166
505	4	1	4	7	2	1	19	3.9471
506	3	1	5	1	1	1	12	4.6664
507	2	1	4	2	1	0	10	1.5500
508	4	0	4	1	3	0	12	7.3332
509	2	2	0	2	1	0	7	2.9463
510	0	0	5	2	0	1	8	4.5000
511	0	2	6	2	4	0	14	5.3570
512	2	2	3	2	2	1	12	0.9997
513	0	1	4	0	0	1	6	4.3332
514	2	3	7	2	2	2	18	1.7774
515	0	2	1	1	1	2	7	3.5534
516	2	1	1	1	1	1	7	2.1426
517	1	0	5	2	0	1	9	3.4164
518	2	2	5	4	1	1	15	1.2664
519	1	1	4	2	1	1	10	1.2000
520	1	1	5	2	0	0	9	3.4888
521	1	0	5	1	2	0	9	4.7498
522	0	2	3	5	0	1	11	2.2498
523	4	1	2	1	2	0	10	7.5500
524	2	2	1	0	1	0	6	4.4165
525	0	2	1	1	1	2	7	3.5534
526	2	1	5	6	2	0	16	1.7500
527	1	1	3	3	1	1	10	0.4000
528	1	0	1	0	1	0	3	2.1655
529	2	1	3	5	1	5	17	5.5878
530	0	2	1	1	1	2	7	3.5534
531	8	5	8	6	3	3	33	5.0604
TOTAL	64	48	124	94	44	36	410	14.1558*

$$\frac{P}{P}\ \sigma^{\prime} \times \frac{P}{P}\ \varphi$$

Mating Number	Porphyrica Male	Female	Total	X^2_1
700	4	4	8	0.0000
701	13	9	22	0.4090
702	8	8	16	0.0000
703	1	3	4	0.2500
704	4	4	8	0.0000
705	7	13	20	1.2500
706	4	3	7	0.0000
707	0	4	4	2.2500
708	5	2	7	0.5714
709	8	7	15	0.0000
710	5	1	6	1.5000
711	8	3	11	1.4544
712	5	6	11	0.0000
713	7	13	20	1.2500
714	9	11	20	0.0500
715	3	6	9	0.4444
716	9	6	15	0.2666
717	6	12	18	1.3888
TOTAL	106	115	221	0.2894

Appendix 3.24

$\frac{N+}{N+}$ ♂ X $\frac{A+}{+P}$ ♀

Mating Number	$\frac{A+}{N+}$ Non-Porphyrica Male	Female	$\frac{N+}{+P}$ Porphyrica Male	Female	Total	X_3^2
800	11	14	11	13	49	0.6593
801	6	7	10	8	31	1.1288
802	8	6	8	14	36	3.9999
803	16	7	4	9	36	8.6665*
804	8	10	10	14	42	1.8094
805	8	7	8	11	34	1.0587
806	4	8	7	5	24	1.6664
807	2	2	4	3	11	0.9998
808	4	9	4	13	30	7.5999
809	2	4	2	2	10	1.2000
810	1	3	3	6	13	3.9229
811	5	5	6	7	23	0.4781
812	6	6	4	5	21	0.5237
813	13	12	9	6	40	3.0000
814	7	7	7	6	27	0.1105
815	4	4	2	2	12	1.3332
816	12	13	10	10	45	0.5998
817	6	8	5	7	26	0.7690
818	4	6	1	6	17	3.9409
819	6	11	3	6	26	5.0767
820	5	8	7	6	26	0.7690
821	8	9	5	8	30	1.1999
822	2	4	1	3	10	2.0000
823	3	4	2	0	9	3.8888
824	1	3	4	4	12	1.9999
825	2	5	1	5	13	3.9229
TOTAL	154	182	138	179	653	7.6857

$\frac{A+}{+P}$ ♂ (A+/N+) × $\frac{N+}{N+}$ ♀ (N+/+P)

Mating Number	Non-Porphyrica Male	Non-Porphyrica Female	Porphyrica Male	Porphyrica Female	Total	X_3^2
850	11	8	12	9	40	1.0000
851	5	3	8	3	19	3.5261
852	18	10	8	6	42	7.9046*
853	11	11	4	16	42	6.9523
854	7	5	3	4	19	1.8419
855	5	7	6	9	27	1.2962
856	3	2	8	3	16	5.5000
857	15	11	8	9	43	2.6742
858	10	1	9	8	28	7.1427
859	11	6	14	12	43	3.2324
860	7	9	3	7	26	2.9229
861	2	1	6	4	13	4.5382
862	5	6	14	3	28	9.9999*
863	3	7	5	4	19	1.8419
864	8	9	13	8	38	1.7893
865	3	3	11	5	22	7.8180
866	10	9	12	8	39	0.8973
867	13	7	5	4	29	7.3702
868	8	6	3	4	21	3.0000
869	7	6	11	8	32	1.7500
870	1	4	4	5	14	2.5713
871	3	1	5	2	11	3.1817
872	8	9	7	5	29	1.4443
873	4	11	7	6	28	3.7142
874	13	5	11	7	36	4.4442
875	11	14	15	7	47	3.2977
876	2	2	6	3	13	3.3075
877	7	4	4	1	16	4.5000
878	8	14	12	10	44	1.8180
879	2	9	11	6	28	6.5713
880	8	8	11	6	33	1.5452
881	2	3	2	4	11	0.9998
882	8	12	5	5	30	4.3999
883	4	10	6	7	27	2.7776
884	8	6	9	4	27	2.1850
885	8	7	6	11	32	1.7500
886	6	6	5	7	24	0.3332
887	2	4	5	4	15	1.2664
888	7	6	4	4	21	1.2856
889	1	6	5	4	16	3.5000
890	5	5	5	4	19	0.1577
891	5	6	6	7	24	0.3332
892	5	4	3	4	16	0.5000
892	5	6	5	5	21	0.1385
893	4	5	5	7	21	0.9047
894	10	10	3	9	32	4.2500
895	7	6	7	7	27	0.1109
897	3	1	4	4	12	1.9999
898	9	11	12	13	45	0.7777
899	8	7	13	12	40	2.6000
TOTAL	336	329	366	314	1345	4.2829

$\frac{R+}{R+}$ ♂ X $\frac{A+}{+P}$ ♀

$\frac{A+}{R+}$ $\frac{R+}{+P}$

Mating Number	Non-Porphyrica		Porphyrica		Total	X^2_3
	Male	Female	Male	Female		
900	5	5	5	7	22	0.5452
901	6	5	10	6	27	11.2500 *
902	5	2	3	4	14	1.4284
TOTAL	16	12	18	17	63	0.9046

$\frac{A+}{+P}$ ♂ x $\frac{R+}{R+}$ ♀

Mating Number	$\frac{A+}{R+}$ Non-Porphyrica Male	Female	$\frac{R+}{+P}$ Porphyrica Male	Female	Total	X^2_3
950	10	7	5	8	30	1.7332
951	12	10	11	12	45	0.2443
952	5	5	5	7	22	0.5452
953	1	6	3	3	13	3.9229
954	6	8	4	7	25	1.4000
955	7	8	6	7	28	0.2556
956	5	7	3	5	20	1.6000
957	8	6	3	6	23	2.2172
958	1	5	5	2	13	3.9229
959	4	5	2	5	16	1.5000
960	6	3	4	2	15	2.3332
961	5	4	3	10	22	5.2725
962	4	4	1	2	11	2.4543
963	1	2	0	2	5	2.2000
964	1	4	2	3	10	2.0000
965	10	4	7	5	26	3.2306
TOTAL	86	88	64	86	324	4.3827

Western		Eastern				
$\frac{R}{R}$		$\frac{R_e}{P_e}$				
R/R_e		R/P_e				
male	female	male	female	Total	X^2_3	P
2	2	1	5	10	3.6000	
10	5	6	4	25	3.2308	
5	5	7	6	23	0.4782	
3	1	8	2	14	8.2857	*
3	3	5	4	15	0.8823	
1	2	2	1	6	0.6671	
2	5	3	4	14	1.4276	
4	9	4	6	23	2.9131	

Generation II

Hybrid		Western				
$\frac{R}{P_e}$		$\frac{R}{R}$				
R/R		R/P_e				
male	female	male	female	Total	X^2_3	P
3	4	6	5	18	1.1112	
5	2	6	4	17	2.0589	
4	3	2	4	13	0.8462	
5	3	3	3	14	0.8571	
2	3	5	5	15	1.8236	

Generation III

Hybrid				Western						
$\frac{R}{P_e}$				$\frac{A}{N}$						
A/R		N/R		A/P_e		N/P_e				
♂	♀	♂	♀	♂	♀	♂	♀	Total	X^2_7	P
2	4	3	2	0	2	6	2	21	6.4337	
3	2	4	3	2	5	1	6	26	5.2308	

CHAPTER FOUR

APPENDICES

Locality and Date of Collection for Each Population Sample

Population	Date	Season	Location & Notes
Hay	27.9.71	Spring	3 miles east on Darlington Point Road. Migrant Adult Population.
Hay	12.4.72	Autumn	2 miles south on Deniliquin Road. Migrant Adult Population.
Boulia	17.5.72	Autumn	Collected and scored in Queensland. by R.A.H. Davies (unpublished).
Coonamble	17.11.60	Spring	10 miles south on Curban Road. Collected and scored by Byrne (1962).
Trangie	29.3.73	Autumn	Trangie Experimental Station.
Coona	28.3.73	Autumn	20 miles north of Wilcannia.
White Cliffs	28.3.73	Autumn	White Cliffs
White Cliffs	13.10.73	Spring	White Cliffs. Progeny of White Cliffs 28.3.73.
Tibooburra	12.10.73	Spring	12 miles south. Progeny of White Cliffs 28.3.73.

Elementary Symmetric Functions Expected for the Genotypes of the various Populations

Population	Sex	Dominant	Frequency	Cumulative	Cumulative Product	Successive Product
HAY	Male	N	432	432		
1972		T	366	798	158,112	
(Autumn)		A	43	841	192,426	6,798,816
					70.5374	0.9136
	Female	N	460	460		
		T	392	852	180,320	
		A	34	886	209,288	6,130,880
					75.2513	0.7927
BOULIA	Male	N	349	349		
1972		T	288	637	100,512	
(Autumn)		A	27	664	117,711	2,713,824
					108.2898	2.2968
	Female	N	400	400		
		T	432	832	172,800	
		A	31	863	198,592	5,356,800
					139.0700	2.6269
COONAMBLE	Male	N	198	198		
1960		T	263	461	52,074	
(Spring)		A	19	480	60,833	989,406
					102.7584	2.8231
	Female	N	146	146		
		T	267	413	38,982	
		A	7	420	41,873	272,874
					75.7197	0.8923
TRANGIE	Male	N	173	173		
1973		T	400	573	69,200	
(Autumn)		A	29	602	85,817	2,006,800
	Female	N	160	160	70.6895	1.3617
		T	124	284	19,840	
		A	16	300	24,384	317,440
					47.8118	1.2203
COONA	Male	N	45	45		
1973		T	149	194	6,705	
(Autumn)		A	11	205	8,839	73,755
					18.7267	0.3311
	Female	N	79	79		
		T	271	350	21,409	
		A	15	365	26,659	321,135
					86.8371	3.4073
WHITE	Male	N	32	32		
CLIFFS		T	37	69	1,184	
1973		A	1	70	1,253	1,184
(Autumn)					3.1169	0.0073
	Female	N	81	81		
		T	273	354	22,113	
		A	16	370	27,777	353,808
					37.0855	0.6307
WHITE	Male	N	38	38		
CLIFFS		T	61	99	2,318	
1973		A	11	110	3,407	25,498
(Spring)					14.5598	0.4657
	Female	N	28	28		
		T	35	63	980	
		A	5	68	1,295	4,900
					11.5625	0.3906
TIBOOBUR-	Male	N	22	22		
RA		T	67	89	1,474	
1973		A	5	94	1,919	7,370
(Spring)					10.6022	0.2249
	Female	N	34	34		
		T	36	70	1,224	
		A	3	73	1,434	3,672
					13.1559	0.3091
WESTERN	Male	N	63	63		
RACE		T	79	142	4,977	
1961					26.9027	
	Female	N	29	29		
		T	76	105	2,204	
					15.9710	
WESTERN	Male	N	19	19		
RACE		T	43	62	817	
1971/72					10.8933	
	Female	N	20	20		
		T	27	47	540	
					6.9230	

Frequency Distribution Expected for the Genotypes of the various populations

		0	1	2	3
HAY 1972 (Autumn)	Male	2728			
		-841	841		
		70.5374	-141.0748	70.5374	
		-0.9136	2.7408	-2.7408	0.9136
		1956.6238	702.6660	67.7966	0.9136
	Female	2781			
		-886	886		
		75.2563	-150.5126	75.2563	
		-0.7927	2.3781	-2.3781	0.7927
		1969.4636	737.8655	72.8782	0.7927
BOULIA 1972 (Autumn)	Male	1087			
		-664	664		
		108.2898	-216.5796	108.2898	
		-2.2968	6.8904	-6.8904	2.2968
		528.9930	454.3108	101.3994	2.2968
	Female	1428			
		-863	863		
		139.0700	-278.1400	139.0700	
		-2.6269	7.8807	-7.8807	2.6269
		701.4431	592.7407	131.1793	2.6269
COONAMBLE 1960 (Spring)	Male	592			
		-480	480		
		102.7584	-205.5168	102.7584	
		-2.8231	8.4693	-8.4693	2.8231
		211.9353	282.9525	94.2891	2.8231
	Female	553			
		-420	420		
		75.7197	-151.4394	75.7197	
		-0.8923	2.6769	-2.6769	0.8923
		207.8274	271.2375	73.0428	0.8923
TRANGIE 1973 (Autumn)	Male	1214			
		-602	602		
		70.6895	-141.3790	70.6895	
		-1.3617	4.0851	-4.0851	1.3617
		681.3278	464.7061	66.6044	1.3617
	Female	510			
		-300	300		
		47.8118	-95.6236	47.8118	
		1.2203	3.6609	-3.6609	1.2203
		256.5915	208.0373	44.1509	1.2203
COONA 1973 (Autumn)	Male	472			
		-205	205		
		18.7267	-37.4534	18.7267	
		-0.3311	0.9933	-0.9933	0.3311
		285.3956	168.5399	17.7334	0.3311
	Female	307			
		-365	365		
		86.8371	-173.6742	86.8371	
		-3.4073	10.2219	-10.2219	3.4073
		25.4298	201.5477	76.6152	3.4073
WHITE CLIFFS 1973 (Autumn)	Male	402			
		-70	70		
		3.1169	-6.2338	3.1169	
		-0.0073	0.0219	-0.0219	0.0073
		335.1096	63.7881	3.0950	0.0073
	Female	749			
		-370	370		
		37.0855	-74.1710	37.0855	
		-0.6307	1.8921	-1.8921	0.6307
		415.4548	297.7211	35.1934	0.6307
WHITE CLIFFS 1973 (Spring)	Male	234			
		-110	110		
		14.5598	-29.1196	14.5598	
		-0.4657	1.3971	-1.3971	0.4657
		138.0941	82.2775	13.1627	0.4657
	Female	112			
		-68	68		
		11.5625	-23.1250	11.5625	
		-0.3906	1.1718	-1.1718	0.3906
		55.1719	46.0468	10.3907	0.3906
TIBOOBURRA 1973 (Spring)	Male	181			
		-94	94		
		10.6022	-21.2044	10.6022	
		-0.2249	0.6747	-0.6747	0.2249
		97.3773	73.4703	9.9275	0.2249
	Female	109			
		-73	73		
		13.1559	-26.3118	13.1559	
		-0.3091	0.9273	-0.9273	0.3091
		48.8468	47.6155	12.2286	0.3091
WESTERN RACE 1961	Male	185			
		-142	142		
		26.9027	-53.8054	26.9027	
		69.9027	88.1946	26.9027	
	Female	138			
		-105	105		
		15.9710	-31.9420	15.9710	
		48.9710	73.0580	15.9710	
WESTERN RACE 1971/72	Male	74			
		-62	62		
		10.8933	-21.7866	10.8933	
		22.8933	40.2134	10.8933	
	Female	78			
		-47	47		
		6.9230	-13.8460	6.9230	
		37.9230	33.1540	6.9230	

Observed versus Expected Comparison of the number of dominants for the various populations.

			Observed (m+x)	Expected (m)	Difference (x)	Chi-square x^2/m
HAY	Male	0	1826	1956.62	130.62	8.72
1972		1	681	702.67	21.67	0.67
(Autumn)		2+3	221	68.10	152.9	343.29
			2728	2727.39		352.68
	Female	0	1821	1969.46	148.46	11.19
		1	724	737.87	13.87	0.26
		2+3	236	73.66	162.34	357.78
			2781	2780.99		369.23
BOULIA	Male	0	111	528.99	417.99	330.28
1972		1	622	454.31	167.69	61.89
(Autumn)		2+3	354	103.68	250.32	604.36
			1087	1086.98		996.53
	Female	0	270	701.44	431.44	265.36
		1	809	592.74	216.26	78.90
		2+3	349	133.81	215.19	346.06
			1428	1427.99		690.32
COONAMBLE	Male	0	140	211.94	71.94	24.41
1960		1	314	282.95	31.05	3.40
(Spring)		2+3	138	97.10	40.90	17.22
			592	591.99		45.03
	Female	0	149	207.83	58.83	16.65
		1	286	271.24	14.76	0.80
		2+3	118	73.93	44.07	26.27
			553	553.00		43.72
TRANGIE	Male	0	582	681.32	99.32	14.47
1973		1	590	464.70	125.30	33.78
(Autumn)		2+3	42	67.96	25.96	9.91
			1214	1213.98		58.16
	Female	0	192	256.59	64.59	16.25
		1	266	208.03	57.97	16.15
		2+3	52	45.37	6.63	0.96
			510	509.99		33.36
COONA	Male	0	257	285.39	28.39	2.82
1973		1	195	168.53	26.47	4.15
(Autumn)		2+3	20	18.06	1.94	0.20
			472	471.98		7.17
	Female	0	146	25.42	120.58	571.97
		1	152	201.54	49.54	12.17
		2+3	9	80.01	71.01	63.02
			307	306.97		657.16
WHITE	Male	0	330	335.10	5.10	0.07
CLIFFS		1	70	63.78	6.22	0.60
1973		2+3	2	3.09	1.09	0.38
(Autumn)			402	401.97		1.05
	Female	0	388	415.45	27.45	1.81
		1	300	297.72	2.28	0.01
		2+3	61	35.82	5.18	17.70
			749	748.99		19.52
WHITE	Male	0	121	138.09	17.09	2.12
CLIFFS		1	78	82.28	4.28	0.22
1973		2+3	35	13.62	21.38	33.56
(Spring)			234	233.99		35.90
TIBOOBUR-	Male	0	84	97.37	13.37	1.84
RA		1	86	73.47	12.53	2.14
1973		2+3	11	10.14	0.86	0.07
(Spring)			181	180.98		4.05
	Female	0	43	48.85	5.85	0.70
		1	47	47.62	0.62	0.01
		2+3	19	12.54	6.46	3.33
			109	109.01		4.04
WESTERN	Male	0	28	69.90	41.90	25.11
RACE		1	104	88.19	15.80	2.83
1961		2	53	26.90	26.10	25.32
			185	184.99		53.26
	Female	0	18	48.97	30.97	19.58
		1	83	73.05	9.95	1.35
		2	37	15.97	21.03	27.69
			138	137.99		48.62
WESTERN	Male	0	11	22.89	11.89	6.17
RACE		1	50	40.21	9.79	2.38
1971/72		2	13	10.89	2.11	0.40
			74	73.99		8.95
	Female	0	7	37.92	30.92	25.21
		1	43	33.15	9.85	2.92
		2	28	6.92	21.08	64.21
			78	77.99		92.34

APPENDIX 4.5

Calculation for the Absence of Compounds (Test for Multiple Allelomorphism)

HAY 1971 (Spring)

	Male	Female
N	951	687
T	826	622
A	455	365
$\sqrt{N}$	30.8382	26.2106
$\sqrt{T}$	28.7402	24.9399
$\sqrt{A}$	21.3307	19.1049
ε	80.9091	70.2554
$-2\sqrt{RR}$	-42.6144	-38.0000
$-\sqrt{n}$	-37.1618	-31.5594
	+1.1329	+0.6960

HAY 1972 (Autumn)

	Male	Female
N	2182	2203
T	2115	2139
A	1862	1845
$\sqrt{N}$	46.1179	46.9362
$\sqrt{T}$	45.9892	46.2494
$\sqrt{A}$	43.1509	42.9535
ε	135.8520	136.1391
$-2\sqrt{RR}$	-85.4636	-85.3464
$-\sqrt{n}$	-41.0488	-40.8167
	+9.3396	+9.9760

WHITE CLIFFS 1973 (Autumn)

	Male	Female
N	362	437
T	367	627
A	331	400
$\sqrt{N}$	19.0262	20.9045
$\sqrt{T}$	19.1572	25.0399
$\sqrt{A}$	18.1934	20.0000
ε	56.3768	65.9444
$-2\sqrt{RR}$	-36.3318	-39.3954
$-\sqrt{n}$	-20.0000	-26.8886
	+0.0450	-0.3396

WHITE CLIFFS 1973 (Spring)

	Male	Female
N	147	70
T	167	75
A	127	56
$\sqrt{N}$	12.1244	8.3666
$\sqrt{T}$	12.9229	8.6603
$\sqrt{A}$	11.2695	7.4834
ε	36.3168	24.5103
$-2\sqrt{RR}$	-22.0000	-14.5602
$-\sqrt{n}$	-14.6629	-7.0000
	-0.3461	+2.9501

BOULIA 1971 (Autumn)

	Male	Female
N	442	653
T	378	675
A	135	291
$\sqrt{N}$	21.0237	25.5538
$\sqrt{T}$	19.4422	25.9807
$\sqrt{A}$	11.6189	17.0587
ε	52.0848	68.5932
$-2\sqrt{RR}$	-21.0712	-32.8632
$-\sqrt{n}$	-27.4954	33.3466
	+ 3.5182	+2.3834

COONAMBLE 1960 (Spring)

	Male	Female
N	263	231
T	321	351
A	150	151
$\sqrt{N}$	16.2172	15.1986
$\sqrt{T}$	17.9164	18.7349
$\sqrt{A}$	12.2474	12.2882
ε	46.3810	46.2217
$-2\sqrt{RR}$	-24.4130	-23.6642
$-\sqrt{n}$	-22.4053	-23.1732
	-0.4373	-0.6157

TIBOOBURRA 1973 (Spring)

	Male	Female
N	102	65
T	148	67
A	88	44
$\sqrt{N}$	10.0995	8.0623
$\sqrt{T}$	12.1656	8.1854
$\sqrt{A}$	9.3809	6.6333
ε	31.6460	22.8810
$-2\sqrt{RR}$	-18.3304	-13.1150
$-\sqrt{n}$	-8.7750	-6.0828
	+4.5406	+3.6832

TRANGIE 1973 (Autumn)

	Male	Female
N	751	339
T	976	301
A	609	202
$\sqrt{N}$	27.4043	18.4119
$\sqrt{T}$	31.2409	17.3493
$\sqrt{A}$	24.6779	14.2126
ε	83.3231	49.9738
$-2\sqrt{RR}$	-48.2493	-27.7128
$-\sqrt{n}$	-34.3220	-21.7944
	+0.7518	+0.4666

COONA 1973 (Autumn)

	Male	Female
N	298	176
T	402	264
A	266	150
$\sqrt{N}$	17.2626	13.2644
$\sqrt{T}$	20.0499	16.2480
$\sqrt{A}$	16.3095	12.2474
ε	53.6220	41.7598
$-2\sqrt{RR}$	-32.0624	-24.1660
$-\sqrt{n}$	-21.3775	-17.3781
	+0.1821	+0.2157

WESTERN RACE 1961

	Male	Female
N	72	36
T	88	83
A	23	18
$\sqrt{N}$	8.4852	6.0000
$\sqrt{T}$	9.3808	9.1104
$\sqrt{A}$	5.2915	4.2426
ε	23.1575	19.3530
$-2\sqrt{RR}$	-10.5830	-8.4852
$-\sqrt{n}$	-12.2882	-10.5830
	+0.2863	+0.2848

WESTERN RACE 1971-72

	Male	Female
N	24	25
T	48	32
A	11	7
$\sqrt{N}$	4.8989	5.0000
$\sqrt{T}$	6.9282	5.6568
$\sqrt{A}$	3.3166	2.6457
ε	15.1437	13.3025
$-2\sqrt{RR}$	-6.6332	-5.2915
$-\sqrt{n}$	-8.0622	-7.2111
	+0.4483	+0.7999

MALE

		Eastern Race			Western Race	
	Experimental Condition	II	III	IV	V	II
Eastern Race	I	1.470	3.2763	4.6888	1.7356	1.4896
	II		3.4160	4.8100	1.8750	1.6159
	III			1.7858	1.4854	1.4385
	IV				3.1217	3.0516
	V					0.0804

FEMALE

		Eastern Race			Western Race	
	Experimental Condition	II	III	IV	V	II
Eastern Race	I	0.6377	4.2256	6.5110	2.7325	1.2929
	II		3.6198	5.9855	2.1278	0.7451
	III			2.7511	1.4123	2.4703
	IV				4.0605	4.8757
	V					1.1573

1.960*

2.576**

3.291***

CHAPTER FIVE

APPENDICES

Appendix 5.1

Raw data

Chromosome	Bivalent Type	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
1	1							20	35	101	245
	2	26	79	58	26	16	39	70	39	14	1
	3	81	2	2	2	11	19	31	10	4	
	5	8	15	10	6	15	9	17	18	6	4
	6	21		5	5	6	5	5	8	7	1
	7	7		1	3	3					7
	8		1	1	1	1	1	1	1	1	1
	9	4	1	3	3	1		2	2		
2	1	2				1	2	8	42	101	254
	2	15	63	60	18	16	31	54	40	9	2
	3	138		2	3	22	37	35	29	10	
	5			4	3	6	4	1	8	1	
	6	17	5	4	4	4	4	3	4	3	1
	7	7		2	2	3					
3	1					1	1	14	37	130	284
	2	10	48	40	14	16	19	37	28	10	
	3	134		2	3	20	37	33	29	10	
	5	2	2		2	2		1	2	1	
	6	15		3	5	7	2	3	7	3	
	7	4			2	2					4
	8	1			1		1		1		
	9	2			1	1	1	1		2	
4	1					1	1	15	44	125	330
	2		37	24	7	9	17	25	18	11	
	3	110	1	1	8	15	25	22	30	8	
	5	1	3		1	2	1		3	1	
	6	8		1	2	5		1	1	5	1
	7	5			2	3					5
5	1	1						10	47	132	385
	2		25	11	4	6	4	11	12	8	1
	3	94		2	5	15	25	19	22	6	1
	5	1	1			2			2		
	6	4		1	1	2		2	1	1	
	7	3			1	2					3
6	1	1						11	42	156	383
	2		13	12	5	4	7	12	9	5	1
	3	85	1	1	8	14	17	14	19	11	
	5		1				1		1		
	6	2	1	1			1	1			
	7	2			1	1					
7	1	3					2	8	51	183	379
	2		3	8	1	1	5	5	4	1	
	3	59			7	8	12	13	14	5	
8	1						1	3	41	160	407
	2		3	3	1			3	3	1	
	3	59		1	4	4	11	18	14	6	1
9	1							5	39	185	456
	2			1			1		3		
	3	17				1	4	7	3	2	
10	1							3	14	122	494
	3	6						2	1	1	
11	1							1	4	118	625